Re: Form 9-3090 - EcoHealth Alliance

Meicher, Lisa K <lmeicher@usgs.gov>

Tue 5/29/2018 1:47 PM

To: Richgels, Katherine L <krichgels@usgs.gov>

Wow! That was quick. I will get the remaining signatures too.

Lisa K. Meicher
Budget Analyst
USGS National Wildlife Health Center
6006 Schroeder Rd
Madison, WI 53711
608-270-2410
fax 608-270-2415
lmeicher@usgs.gov

On Tue, May 29, 2018 at 1:42 PM, Richgels, Katherine <krichgels@usgs.gov> wrote:

> Ok, great. We need this form to then go to Jonathan and on to Leon (I believe). Can you usher it through the process for me?
>
> Thanks,
> Katie
>
> On Tue, May 29, 2018 at 1:40 PM, Ethics Office, GS-O <ethicsoffice@usgs.gov> wrote:
>
> > Hello,
> >
> > Please find the signed form attached.
> >
> > Please let me know if you need anything else.
> >
> > Thanks,
> > Liz
> >
> > USGS Ethics Office
> >
> > ~~~~~~~~~~~~~
> > U.S. Geological Survey

> 12210 Sunrise Valley Drive, MS 603
> Reston, VA 20192
> EthicsOffice@usgs.gov
> USGS Ethics Office website: www2.usgs.gov/quality_integrity/ethics
>
> On Tue, May 29, 2018 at 2:20 PM, Meicher, Lisa <lmeicher@usgs.gov> wrote:
>> Please review and sign the attached Form 9-3090.
>>
>> Thanks!
>> Lisa
>>
>> Lisa K. Meicher
>> Budget Analyst
>> USGS National Wildlife Health Center
>> 6006 Schroeder Rd
>> Madison, WI 53711
>> 608-270-2410
>> fax 608-270-2415
>> lmeicher@usgs.gov

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Katherine L. D. Richgels, Ph.D.
Branch Chief, Applied Wildlife Health Research
Responsible Official, Federal Select Agent Program
USGS National Wildlife Health Center
6006 Schroeder Rd
Madison, WI 53711
(608) 270 - 2450 (office)
(608) 381 - 2492 (cell)
(608) 270 - 2415 (fax)
krichgels@usgs.gov
www.nwhc.usgs.gov

Form 9-3090
(June 2016 - Supersedes all previous versions)
INTERIM FORM FOR USE WHILE REVISING SHAREPOINT VERSION

NOTE – This form along with SM 500.18 and SM 205.13 are in the review process before becoming final policy adjustments. There is a need to utilize this form now in order to obtain the information necessary to proceed in approving Grant applications. This form will be updated per the comments received from the afore mentioned review. Upon completion of the review process the 9-3090 will be developed into a Sharepoint form which will reduce its length and offer ease in its utilization.

6. *Complete this section only if USGS is applying to RFP as a Subawardee, else skip to next section*

Original Source of Funding Being Awarded to the Grant Source

Legal Name of Original Source of Funds being provided to Grant Source	Department of Defense (DoD), Defense Advanced Research Projects Agency (DARPA), Preventing Emerging Pathogenic Threats (PREEMPT)
Short Name or Acronym	PREEMPT
Address	675 N. Randolph St
Phone Number	DARPA-SN-18-18@darpa.mil
Contact Name	Dr. Jim Gimlett, Program Manager
Website of Original Source	https://www.darpa.mil/
Website of RFP	https://www.fbo.gov/index?s=opportunity&mode=form&id=4e14aa2d9a172c92a41d8fc181128435&tab=core&_cview=0

Make a Selection: ☐ University ☐ Non-Profit ☐ State/Local Governmental Unit
☐ Individual ☐ For Profit ☒ Federal Governmental Unit

International Entity? ☐ Yes ☒ No

Private Corporation? ☐ Yes ☒ No

State of Incorporation (if applicable)	

If a corporation, is it a subsidiary of a larger entity? ☐ Yes ☐ No ☒ NA

If Yes,

Identify the Parent Company	
Website of Parent Company	

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7. USGS Office Submitting Grant Information

Principal Investigator (PI) Name	Tonie Rocke
PI Title	Epizootiologist
PI Phone Number	608-270-2451
PI Email	trocke@usgs.gov

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Request to Apply for a Grant Funds-In Award

(See Appendix A for Instructions for completion of Form 9-3090)

1. Type of Award: ☐ Prime Award ☒ Subaward

2. Provide documentation confirming USGS can accept the funds as either a prime or sub-awardee.
   We can accept funds for this RFP using the authority in 31 U.S.C. 1535A Economy Act.

3. Grant Application Deadline

3/27/2018

4. Projected Grant Award Date

12/1/2018

5. Grant Source Information (Entity from whom USGS is directly receiving awarded funds)

Name	EcoHealth Alliance
Address	460 W. 34th St, 17th Floor
Phone Number	212-380-4474
Contact Name	Dr. Peter Daszak
Website of Grant Source	
Web Link to RFP	

Make a Selection: ☐ University ☒ Non-Profit ☐ State/Local Governmental Unit
☐ Individual ☐ For Profit

International Entity? ☐ Yes ☒ No

Private Corporation? ☐ Yes ☒ No

State of Incorporation (if applicable)	

If a corporation, is it a subsidiary of a larger entity? ☐ Yes ☐ No ☒ NA

If Yes,

Identify the Parent Company	
Website of Parent Company	

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Name of Science Center	National Wildlife Health Center
List Other USGS Science Team Members Include Name, Phone, Email, Center	N/A

8. Science Center/Cost Center Director (Manager)

Name of Director	Jonathan Sleeman
Title	Center Director
Phone Number	608-270-2401
Email	jsleeman@usgs.gov
Name of Science Center	National Wildlife Health Center
Organization Code of Center	GGEMNC0000
Administrative Officer (AO)	Thomas Hankins, thankins@usgs.gov

9. Estimated Dollar Value of Requested Grant (funds coming into USGS)

847,292.29

10. Briefly Describe the Proposed Project or attach an SOW if applicable at this time

See attached proposal.

11. Explain how this grant will support a USGS mission or project

The application and validation of the technique outlined in this project will also be applied to on-going development of a white-nose syndrome vaccine for bats. Managing white-nose syndrome in bat populations is a priority for the NWHC, Ecosystems Mission Area, and the U.S. Fish and Wildlife Service.

12. Does Grant solicitation/award limit Center from charging its full overhead? ☐ Yes ☒ No
If Yes,

Explain the Limitation	
Contact Center AO to discuss whether the limitation meets Bureau established accounting practices.	

13. Do you intend to provide an out-going sub-award of grant funds received through either a contract or financial assistance (cooperative agreement)? ☐ Yes ☒ No

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If Yes,

Briefly Explain the Out-Going Sub-Award Plan	
Contact Center AO for review of sub-award plans and appropriateness of sub-award in consultation with OAG as necessary.	

14. Is there a Grant Agreement template associated with the RFP that the providing entity is requesting be utilized in the award of funds? ☐ Yes ☒ No

If No,	**Start agreement negotiations utilizing the Technical Assistance Agreement template found on the Office of Policy and Analysis site at https://insight.usgs.gov/aei/offices/oa/opa/SitePages/Home.aspx**
If Yes,	**Contact Center AO for review of Grant award template and appropriateness of agreement language.**

15. Does the grant require project reporting and/or special invoicing? ☐ Yes ☐ No ☒ Unknown

If Yes,

Describe any concerns the submitting office/PI has regarding the project reporting or invoicing	

16. Does the grant have special auditing requirements? ☐ Yes ☐ No ☒ Unknown

If Yes,

Describe the audit requirements	

17. Does the Grant contain Intellectual Property (IP) conditions and provisions?
☐ Yes ☐ No ☒ Unknown

If Yes,

Does the Grant require a license to new IP?	
Does the Grant require assignment of IP?	
Is there background IP of either party used to accomplish the SOW?	
Is a party identified who will take the lead when new IP is made?	

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If yes, on any of the above, please contact Center AO for review of compliance with Bureau IP policy. Patents and Licensing: http://internal.usgs.gov/ops/opa/index.html

18. Does the Grant RFP contain restrictions on data use rights or access to research results?
☐ Yes ☒ No ☐ Unknown
If Yes,

Are there confidentiality terms for protection of the data or research and development information? If yes, explain.	
Are there data ownership terms? If yes, explain.	
Are there access terms and conditions? If yes, explain.	
Is a data management or distribution plan required? If yes, explain.	
If yes on any of the above, please contact Center AO for review of compliance with Bureau policy and consultation with the Office of Policy and Analysis.	

19. Does the Grant RFP contain restrictions on preparation or submission of publications or reports?
☐ Yes ☒ No ☐ Unknown
If Yes,

Is a review by the Grantor required prior to publication? If yes, explain.	
Is there a request for naming entity as support or as designated author? If yes, explain.	
If yes on any of the above, please contact Center AO for review of compliance with OSQI policy related to the Fundamental Science Practices and the Office of	

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Communications for guidance related publications and copyrights.

20. Business, Contract or Litigation Relationships

A. USGS Center	**B. Principal Investigator (PI)**
(1) Is there a Purchase Contract with the Grant Source? ☐ Yes ☒ No	(1) Are you involved with any type of project involving the Grant Source? ☐ Yes ☒ No
(2) Is there an existing CRADA with the Grant Source? ☐ Yes ☒ No	(2) Are you involved in development of a prospective CRADA with Grant Source? ☐ Yes ☒ No
(3) Is there a Cooperative/State/Local Agreement with the Grant Source? ☐ Yes ☒ No	(3) Do you have any type of financial interest in the Grant Source? ☐ Yes ☒ No
(4) Are you aware of any litigation pending or anticipated with the Grant Source? ☐ Yes ☒ No	
(5) Is the Grant Source listed in the current Financial Guide for USGS Employees Interests? http://www.usgs.gov/quality_integrity/ethics ☐ Yes ☒ No	

If the answer is 'Yes' to any of the questions in the above section please provide details below: (Attach additional sheets if necessary)

Question: ☐ (Input Question you are answering, i.e. 1(a), 1(b), etc.)

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Question: ☐ (Input Question you are answering, i.e. 1(a), 1(b), etc.)

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Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

[ ]

Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

[ ]

Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

[ ]

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SIGNATURE/CERTIFICATION SECTION

I. CERTIFICATION AND CONFLICT OF INTEREST
This section to be completed by USGS employee applying for competitive grant

I have completed the above information and certify they are accurate to the best of my knowledge and belief. The activities proposed by the competitive grant will not conflict with my responsibilities to USGS, and my responsibility to report government inventions. I further certify that to the best of my knowledge neither I, nor my spouse, child, parent, sibling nor any organization in which I/we serve as officer, director, trustee or employee:

i) holds financial interest in the above entity;
ii) has or will receive a gift or gratuity from the above entity or any entity that has a substantial interest in the preparation, negotiation or approval of my competitive grant.

I understand that if the facts change during the term of any grant that I may receive, I have an obligation to advise my Supervisor and the Ethics Office at ethicsoffice@usgs.gov in writing.

Principal Investigator Tonie Rocke

Signature: Tonie Rocke Date: 4/18/2018

II. ADMINISTRATIVE CERTIFICATION

I have reviewed the RFP associated with this Grant. ☐ Yes ☐ No

Answers provided above are determined to be accurate and made to the best of individual ability based on the information available at this time.

Administrative Officer: T Morris Hankins Jr

Signature: [signature] Date: 18 APR 2018

III. ETHICS OFFICE DETERMINATION
This section to be completed by USGS Ethics Office

☒ Based upon the information presented in this form, no prohibited source or conflict of interest issues have been identified.

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☐ Based upon the information presented in this form, prohibited source or conflict of interest issues have been identified.

Comments *(optional)*

Signature: E D Bacca Elizabeth D. Bacca Associate Ethics Counselor Date: 05.29.2018

IV. CENTER DIRECTOR DETERMINATION
This section to be completed by Center Director/Manager

This application for grant meets USGS policy and procedures, as described in SM 500.18. Based on the information presented in this form, I have evaluated this grant and determined it is acceptable to proceed with the application for a grant.

☐ Yes ☐ No

Comments *(optional)*

Signature: T Sleeman Date: 6/6/18

V. ASSOCIATE/REGIONAL DIRECTOR DETERMINATION
When applicable given thresholds in the SM 500.18 and SM 205.13
SM Drafts out for Comments due 7/15/16

This application for grant meets USGS policy and procedures, as described in SM 500.18. Based on the information presented in this form, I have evaluated this grant and determined it is acceptable to proceed with the application for a grant.

☐ Yes ☐ No

Comments *(optional)*

Signature: _____ Date: _____

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INSTRUCTIONS for Completing a Request to Apply for a Grant Funds-In Award, Form 9-3090

Purpose: The purpose of the form is to determine if it is appropriate for USGS to apply to Requests for Proposals (RFP) publicly announced and open to Federal entities, and whether USGS policies and procedures allow the project to proceed as outlined/envisioned.

Prior to completing Form 9-3090, a determination of whether the funding it is applicable as an incoming Grant is needed. Guidance on applicability can be found in Survey Manual 500.18 (current draft is out for review) and the Policy and Grant Handbook (under construction).

While SM 500.18 is in Draft form, the following definitions are provided for guidance:
See Definition of a Grant in section 1 below. In addition, the funding is not considered a grant when the following situations are present:

1) When Federal entities invite only other Federal entities to apply for a grant.
2) When there is an internal USGS competition for funds.
3) If an entity requests the USGS submit a proposal for a science effort, but it is not in a competitive situation (even if the providing entity calls the document a grant).
4) When USGS enters into a reimbursable agreement with an entity that has been awarded a grant where the USGS was not specifically involved or identified in the proposal and/or competition.

Blocks on the Form:

1. Definitions for Prime Award and Subaward can be found in Survey Manual 500.18. For additional information and discussion, see the Grant Handbook.

   While SM 500.18 is in Draft form, the following definitions are provided for guidance:

   Grant. A grant is a program in which the grant-making entity makes funds or other resources available to eligible participants through a competitive process. A grant, if awarded by a grant-making entity, may be awarded directly or indirectly to the USGS. For instructional guidance and forms specific to incoming grants, see Grant Application and Acceptance Handbook 500.18-H. Financial management procedures are outlined in the Financial Operating Procedures Handbook (FOP).

   (1) Direct Grant (commonly referred to as a "prime award") is one in which the USGS receives the grant funding as a prime recipient.
   (2) Indirect grant (commonly referred as a "subaward") is one in which the USGS has collaborated with an entity to submit a proposal as a co-Principal Investigator (co-PI).
2. Documentation used to confirm that USGS can accept the grant funds might be written language from the RFP itself, an email from the prime award entity indicating that USGS can be a sub-awardee or something from the Grants source.
3. Enter the date the Request For Proposal (RFP) closes.
4. Enter the date it is anticipated the Grant will be awarded.
5. Enter information specific to the Entity from whom USGS is directly receiving awarded funds.
6. Enter information specific to the Original source of funds if USGS is applying as a sub-awardee.
7. Enter Principal Investigator (PI) and Co-PI specific information.
8. Enter Center Director/Manager and Center information.
9. Enter dollar amount of USGS specific requested funds.
10. Provide a brief description of the proposed project or attach an SOW if one has been prepared.
11. Provide an explanation of how the grant will support a USGS mission or project.

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12. Identify whether the Grant has an overhead limitation. If there is a limitation, provide a narrative explanation. For information on overhead structure, management and flexibilities, see Financial Operating Procedures Handbook Chapters 5, 6 and 7.
13. Provide information on intentions for out-going contracts or financial assistance agreements. These will be handled by the Office of Acquisitions and Grants (OAG).
https://insight.usgs.gov/aei/offices/oa/oag/AOP/introduction.pdf
14. Determine if there is a pre-identified agreement template from the granting entity to be used. If so, guidance can be provided by the Office of Policy and Analysis after consultation with the Center's Administrative Officer. A USGS Grant Agreement template found at https://insight.usgs.gov/aei/offices/oa/opa/SitePages/Home.aspx if we have the opportunity to initiate the agreement.
15. Describe any special requirements on project reporting and/or invoicing. Discuss these requirements with Administrative Officer to ensure they can be implemented.
16. Outline special auditing requirements identified in the RFP if applicable.
17. Outline any Intellectual Property (IP) provisions or expectations for this project. Additional information on IP can be accessed at Patents and Licensing: http://internal.usgs.gov/ops/opa/index.html.
18. Outline any restrictions or expectations on data use rights and access to research results. Additional information on these topics can be obtained from the OPA at GS-AEI_opa@usgs.gov.
19. Outline restrictions on preparation or submission of publications or reports. Additional information on these topics can be obtained from OSQI related to the Fundamental Science Practices (FSP) and the Office of Communications for guidance related publications and copyrights. FSP guidelines can be found at https://www2.usgs.gov/fsp/.
20. Answer the questions from the perspective of both the local USGS Center and the PI. Answers will be considered in determining a conflict of interest or appearance thereof. Any "Yes" answers will require an explanation.

Signature/Certification Section:

I. The Principal Investigator signs this section certifying per the statement provided. Upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Administrative Officer for review and signature.

II. The Administrative Officer signs this section certifying per the statement provided. Upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Ethics Office for review and signature.

III. The Ethics Office signs this section certifying per the statement provided. Upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Center Director/Manager for review and signature.

IV. The Center Director/Manager signs this section certifying per the statement provided in accordance with SM 500.18. This may be the final signature if it is under the thresholds outlined in the Delegations of Authority, SM 205.13. If additional approval is needed, upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Associate/Regional Director (AD/RD) for final review and signature.

V. The AD/RD signs this section certifying per the statement provided in accordance with SM 500.18. Upon signature the signed form will be returned to the Center Director/Manager filing. Upon award of the Grant this 9-3090 must be part of the Agreement packet that is submitted for OPA Review at GS-AEI_opa@usgs.gov.

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- Associate Directors and Regional Directors – grants from Federal, State, and local governments of $750,000 or more, and grants of $250,000 or more from sources other than Federal, State, and local governments.
- Science Center Directors and Cost Center Managers – grants from Federal, State, and local governments of less than $750,000, and grants less than $250,000 from sources other than Federal, State, and local governments.

Form 9-3090 - EcoHealth Alliance

Meicher, Lisa K <lmeicher@usgs.gov>

Tue 5/29/2018 1:20 PM

To: Ethics Office, GS-O <ethicsoffice@usgs.gov>
Cc: Richgels, Katherine L <krichgels@usgs.gov>

Please review and sign the attached Form 9-3090.

Thanks!
Lisa

Lisa K. Meicher
Budget Analyst
USGS National Wildlife Health Center
6006 Schroeder Rd
Madison, WI 53711
608-270-2410
fax 608-270-2415
lmeicher@usgs.gov

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   We can accept funds for this RFP using the authority in 31 U.S.C. 1535A Economy Act.

3. Grant Application Deadline

3/27/2018

4. Projected Grant Award Date

12/1/2018

5. Grant Source Information (Entity from whom USGS is directly receiving awarded funds)

Name	EcoHealth Alliance
Address	460 W. 34th St, 17th Floor
Phone Number	212-380-4474
Contact Name	Dr. Peter Daszak
Website of Grant Source	
Web Link to RFP	

Make a Selection: ☐ University ☒ Non-Profit ☐ State/Local Governmental Unit
☐ Individual ☐ For Profit

International Entity? ☐ Yes ☒ No

Private Corporation? ☐ Yes ☒ No

State of Incorporation (if applicable)	

If a corporation, is it a subsidiary of a larger entity? ☐ Yes ☐ No ☒ NA

If Yes,

Identify the Parent Company	
Website of Parent Company	

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6. *Complete this section only if USGS is applying to RFP as a Subawardee, else skip to next section*

Original Source of Funding Being Awarded to the Grant Source

Legal Name of Original Source of Funds being provided to Grant Source	Department of Defense (DoD), Defense Advanced Research Projects Agency (DARPA), Preventing Emerging Pathogenic Threats (PREEMPT)
Short Name or Acronym	PREEMPT
Address	675 N. Randolph St
Phone Number	DARPA-SN-18-18@darpa.mil
Contact Name	Dr. Jim Gimlett, Program Manager
Website of Original Source	https://www.darpa.mil/
Website of RFP	https://www.fbo.gov/index?s=opportunity&mode=form&id=4e14aa2d9a172c92a41d8fc181128435&tab=core&_cview=0

Make a Selection: ☐ University ☐ Non-Profit ☐ State/Local Governmental Unit
☐ Individual ☐ For Profit ☒ Federal Governmental Unit

International Entity? ☐ Yes ☒ No

Private Corporation? ☐ Yes ☒ No

State of Incorporation (if applicable)	

If a corporation, is it a subsidiary of a larger entity? ☐ Yes ☐ No ☒ NA

If Yes,

Identify the Parent Company	
Website of Parent Company	

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7. USGS Office Submitting Grant Information

Principal Investigator (PI) Name	Tonie Rocke
PI Title	Epizootiologist
PI Phone Number	608-270-2451
PI Email	trocke@usgs.gov

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Name of Science Center	National Wildlife Health Center
List Other USGS Science Team Members Include Name, Phone, Email, Center	N/A

8. Science Center/Cost Center Director (Manager)

Name of Director	Jonathan Sleeman
Title	Center Director
Phone Number	608-270-2401
Email	jsleeman@usgs.gov
Name of Science Center	National Wildlife Health Center
Organization Code of Center	GGEMNC0000
Administrative Officer (AO)	Thomas Hankins, thankins@usgs.gov

9. Estimated Dollar Value of Requested Grant (funds coming into USGS)

847,292.29

10. Briefly Describe the Proposed Project or attach an SOW if applicable at this time

See attached proposal.

11. Explain how this grant will support a USGS mission or project

The application and validation of the technique outlined in this project will also be applied to on-going development of a white-nose syndrome vaccine for bats. Managing white-nose syndrome in bat populations is a priority for the NWHC, Ecosystems Mission Area, and the U.S. Fish and Wildlife Service.

12. Does Grant solicitation/award limit Center from charging its full overhead? ☐ Yes ☒ No
If Yes,

Explain the Limitation	
Contact Center AO to discuss whether the limitation meets Bureau established accounting practices.	

13. Do you intend to provide an out-going sub-award of grant funds received through either a contract or financial assistance (cooperative agreement)? ☐ Yes ☒ No

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If Yes,

Briefly Explain the Out-Going Sub-Award Plan	
Contact Center AO for review of sub-award plans and appropriateness of sub-award in consultation with OAG as necessary.	

14. Is there a Grant Agreement template associated with the RFP that the providing entity is requesting be utilized in the award of funds? ☐ Yes ☒ No

If No,	**Start agreement negotiations utilizing the Technical Assistance Agreement template found on the Office of Policy and Analysis site at https://insight.usgs.gov/aei/offices/oa/opa/SitePages/Home.aspx**
If Yes,	**Contact Center AO for review of Grant award template and appropriateness of agreement language.**

15. Does the grant require project reporting and/or special invoicing? ☐ Yes ☐ No ☒ Unknown

If Yes,

Describe any concerns the submitting office/PI has regarding the project reporting or invoicing	

16. Does the grant have special auditing requirements? ☐ Yes ☐ No ☒ Unknown

If Yes,

Describe the audit requirements	

17. Does the Grant contain Intellectual Property (IP) conditions and provisions?
☐ Yes ☐ No ☒ Unknown

If Yes,

Does the Grant require a license to new IP?	
Does the Grant require assignment of IP?	
Is there background IP of either party used to accomplish the SOW?	
Is a party identified who will take the lead when new IP is made?	

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If yes, on any of the above, please contact Center AO for review of compliance with Bureau IP policy. Patents and Licensing: http://internal.usgs.gov/ops/opa/index.html

18. Does the Grant RFP contain restrictions on data use rights or access to research results?
☐ Yes ☒ No ☐ Unknown
If Yes,

Are there confidentiality terms for protection of the data or research and development information? If yes, explain.	
Are there data ownership terms? If yes, explain.	
Are there access terms and conditions? If yes, explain.	
Is a data management or distribution plan required? If yes, explain.	
If yes on any of the above, please contact Center AO for review of compliance with Bureau policy and consultation with the Office of Policy and Analysis.	

19. Does the Grant RFP contain restrictions on preparation or submission of publications or reports?
☐ Yes ☒ No ☐ Unknown
If Yes,

Is a review by the Grantor required prior to publication? If yes, explain.	
Is there a request for naming entity as support or as designated author? If yes, explain.	
If yes on any of the above, please contact Center AO for review of compliance with OSQI policy related to the Fundamental Science Practices and the Office of	

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(June 2016 - Supersedes all previous versions)
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Communications for guidance related publications and copyrights.

20. Business, Contract or Litigation Relationships

A. USGS Center	**B. Principal Investigator (PI)**
(1) Is there a Purchase Contract with the Grant Source? ☐ Yes ☒ No	(1) Are you involved with any type of project involving the Grant Source? ☐ Yes ☒ No
(2) Is there an existing CRADA with the Grant Source? ☐ Yes ☒ No	(2) Are you involved in development of a prospective CRADA with Grant Source? ☐ Yes ☒ No
(3) Is there a Cooperative/State/Local Agreement with the Grant Source? ☐ Yes ☒ No	(3) Do you have any type of financial interest in the Grant Source? ☐ Yes ☒ No
(4) Are you aware of any litigation pending or anticipated with the Grant Source? ☐ Yes ☒ No	
(5) Is the Grant Source listed in the current Financial Guide for USGS Employees Interests? http://www.usgs.gov/quality_integrity/ethics ☐ Yes ☒ No	

If the answer is 'Yes' to any of the questions in the above section please provide details below: (Attach additional sheets if necessary)

Question: ☐ (Input Question you are answering, i.e. 1(a), 1(b), etc.)

Question: ☐ (Input Question you are answering, i.e. 1(a), 1(b), etc.)

Question: ☐ (Input Question you are answering, i.e. 1(a), 1(b), etc.)

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Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

[ ]

Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

[ ]

Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

[ ]

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SIGNATURE/CERTIFICATION SECTION

I. CERTIFICATION AND CONFLICT OF INTEREST
This section to be completed by USGS employee applying for competitive grant

I have completed the above information and certify they are accurate to the best of my knowledge and belief. The activities proposed by the competitive grant will not conflict with my responsibilities to USGS, and my responsibility to report government inventions. I further certify that to the best of my knowledge neither I, nor my spouse, child, parent, sibling nor any organization in which I/we serve as officer, director, trustee or employee:

i) holds financial interest in the above entity;
ii) has or will receive a gift or gratuity from the above entity or any entity that has a substantial interest in the preparation, negotiation or approval of my competitive grant.

I understand that if the facts change during the term of any grant that I may receive, I have an obligation to advise my Supervisor and the Ethics Office at ethicsoffice@usgs.gov in writing.

Principal Investigator Tonie Rocke

Signature: Tonie E Rocke Date: 4/18/2018

II. ADMINISTRATIVE CERTIFICATION

I have reviewed the RFP associated with this Grant. ☐ Yes ☐ No

Answers provided above are determined to be accurate and made to the best of individual ability based on the information available at this time.

Administrative Officer: I THOMAS HENKINS JR

Signature: [signature] Date: 18 APR 2018

III. ETHICS OFFICE DETERMINATION
This section to be completed by USGS Ethics Office

☐ Based upon the information presented in this form, no prohibited source or conflict of interest issues have been identified.

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☐ Based upon the information presented in this form, prohibited source or conflict of interest issues have been identified.

Comments *(optional)*

Signature: _____ Date: _____

IV. CENTER DIRECTOR DETERMINATION
This section to be completed by Center Director/Manager

This application for grant meets USGS policy and procedures, as described in SM 500.18. Based on the information presented in this form, I have evaluated this grant and determined it is acceptable to proceed with the application for a grant.

☐ Yes ☐ No

Comments *(optional)*

Signature: _____ Date: _____

V. ASSOCIATE/REGIONAL DIRECTOR DETERMINATION
When applicable given thresholds in the SM 500.18 and SM 205.13
SM Drafts out for Comments due 7/15/16

This application for grant meets USGS policy and procedures, as described in SM 500.18. Based on the information presented in this form, I have evaluated this grant and determined it is acceptable to proceed with the application for a grant.

☐ Yes ☐ No

Comments *(optional)*

Signature: _____ Date: _____

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INSTRUCTIONS for Completing a Request to Apply for a Grant Funds-In Award, Form 9-3090

Purpose: The purpose of the form is to determine if it is appropriate for USGS to apply to Requests for Proposals (RFP) publicly announced and open to Federal entities, and whether USGS policies and procedures allow the project to proceed as outlined/envisioned.

Prior to completing Form 9-3090, a determination of whether the funding it is applicable as an incoming Grant is needed. Guidance on applicability can be found in Survey Manual 500.18 (current draft is out for review) and the Policy and Grant Handbook (under construction).

While SM 500.18 is in Draft form, the following definitions are provided for guidance:
See Definition of a Grant in section 1 below. In addition, the funding is not considered a grant when the following situations are present:

1) When Federal entities invite only other Federal entities to apply for a grant.
2) When there is an internal USGS competition for funds.
3) If an entity requests the USGS submit a proposal for a science effort, but it is not in a competitive situation (even if the providing entity calls the document a grant).
4) When USGS enters into a reimbursable agreement with an entity that has been awarded a grant where the USGS was not specifically involved or identified in the proposal and/or competition.

Blocks on the Form:

1. Definitions for Prime Award and Subaward can be found in Survey Manual 500.18. For additional information and discussion, see the Grant Handbook.

   While SM 500.18 is in Draft form, the following definitions are provided for guidance:

   Grant. A grant is a program in which the grant-making entity makes funds or other resources available to eligible participants through a competitive process. A grant, if awarded by a grant-making entity, may be awarded directly or indirectly to the USGS. For instructional guidance and forms specific to incoming grants, see Grant Application and Acceptance Handbook 500.18-H. Financial management procedures are outlined in the Financial Operating Procedures Handbook (FOP).

   (1) Direct Grant (commonly referred to as a "prime award") is one in which the USGS receives the grant funding as a prime recipient.
   (2) Indirect grant (commonly referred as a "subaward") is one in which the USGS has collaborated with an entity to submit a proposal as a co-Principal Investigator (co-PI).
2. Documentation used to confirm that USGS can accept the grant funds might be written language from the RFP itself, an email from the prime award entity indicating that USGS can be a sub-awardee or something from the Grants source.
3. Enter the date the Request For Proposal (RFP) closes.
4. Enter the date it is anticipated the Grant will be awarded.
5. Enter information specific to the Entity from whom USGS is directly receiving awarded funds.
6. Enter information specific to the Original source of funds if USGS is applying as a sub-awardee.
7. Enter Principal Investigator (PI) and Co-PI specific information.
8. Enter Center Director/Manager and Center information.
9. Enter dollar amount of USGS specific requested funds.
10. Provide a brief description of the proposed project or attach an SOW if one has been prepared.
11. Provide an explanation of how the grant will support a USGS mission or project.

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12. Identify whether the Grant has an overhead limitation. If there is a limitation, provide a narrative explanation. For information on overhead structure, management and flexibilities, see Financial Operating Procedures Handbook Chapters 5, 6 and 7.
13. Provide information on intentions for out-going contracts or financial assistance agreements. These will be handled by the Office of Acquisitions and Grants (OAG). https://insight.usgs.gov/aei/offices/oa/oag/AOP/introduction.pdf
14. Determine if there is a pre-identified agreement template from the granting entity to be used. If so, guidance can be provided by the Office of Policy and Analysis after consultation with the Center's Administrative Officer. A USGS Grant Agreement template found at https://insight.usgs.gov/aei/offices/oa/opa/SitePages/Home.aspx if we have the opportunity to initiate the agreement.
15. Describe any special requirements on project reporting and/or invoicing. Discuss these requirements with Administrative Officer to ensure they can be implemented.
16. Outline special auditing requirements identified in the RFP if applicable.
17. Outline any Intellectual Property (IP) provisions or expectations for this project. Additional information on IP can be accessed at Patents and Licensing: http://internal.usgs.gov/ops/opa/index.html.
18. Outline any restrictions or expectations on data use rights and access to research results. Additional information on these topics can be obtained from the OPA at GS-AEI_opa@usgs.gov.
19. Outline restrictions on preparation or submission of publications or reports. Additional information on these topics can be obtained from OSQI related to the Fundamental Science Practices (FSP) and the Office of Communications for guidance related publications and copyrights. FSP guidelines can be found at https://www2.usgs.gov/fsp/.
20. Answer the questions from the perspective of both the local USGS Center and the PI. Answers will be considered in determining a conflict of interest or appearance thereof. Any "Yes" answers will require an explanation.

Signature/Certification Section:

I. The Principal Investigator signs this section certifying per the statement provided. Upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Administrative Officer for review and signature.

II. The Administrative Officer signs this section certifying per the statement provided. Upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Ethics Office for review and signature.

III. The Ethics Office signs this section certifying per the statement provided. Upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Center Director/Manager for review and signature.

IV. The Center Director/Manager signs this section certifying per the statement provided in accordance with SM 500.18. This may be the final signature if it is under the thresholds outlined in the Delegations of Authority, SM 205.13. If additional approval is needed, upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Associate/Regional Director (AD/RD) for final review and signature.

V. The AD/RD signs this section certifying per the statement provided in accordance with SM 500.18. Upon signature the signed form will be returned to the Center Director/Manager filing. Upon award of the Grant this 9-3090 must be part of the Agreement packet that is submitted for OPA Review at GS-AEI_opa@usgs.gov.

While SM 500.18 is in Draft form, the following signature thresholds are provided for guidance:

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- Associate Directors and Regional Directors – grants from Federal, State, and local governments of $750,000 or more, and grants of $250,000 or more from sources other than Federal, State, and local governments.
- Science Center Directors and Cost Center Managers – grants from Federal, State, and local governments of less than $750,000, and grants less than $250,000 from sources other than Federal, State, and local governments.

Re: [EXTERNAL] RE: DEFUSE documents as submitted

Richgels, Katherine L <krichgels@usgs.gov>

Wed 3/28/2018 1:01 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Thanks Tonie, I appreciate the support. I can't find a final proposal email from Luke, can you forward it when you get a chance?

Katie

Sent from my Verizon, Samsung Galaxy smartphone

-------- Original message --------
From: "Rocke, Tonie" <trocke@usgs.gov>
Date: 3/28/18 1:47 PM (GMT-05:00)
To: Katherine Richgels <krichgels@usgs.gov>
Subject: Re: [EXTERNAL] RE: DEFUSE documents as submitted

I believe Luke Hamel sent you all the documents including our budget. By the way, thanks for your letter; it was thoughtful of you, and rest assured, I am very supportive of you in this position (despite my initial hesitance which I now regret). I have had 3 branch chiefs during my term here, and you are by far the best! Keep up the good work. It will get easier. Best - Tonie

On Wed, Mar 28, 2018 at 12:30 PM, Katherine Richgels <krichgels@usgs.gov> wrote:

> Congrats! Can you send me a copy if the submitted proposal?
>
> Thanks,
> Katie
>
> Sent from my Verizon, Samsung Galaxy smartphone
>
> -------- Original message --------
> From: "Rocke, Tonie" <trocke@usgs.gov>
> Date: 3/28/18 1:18 PM (GMT-05:00)
> To: Peter Daszak <daszak@ecohealthalliance.org>
> Cc: Luke Hamel <hamel@ecohealthalliance.org>, Alison Andre <andre@ecohealthalliance.org>, Rachel Abbott <rabbott@usgs.gov>, "Richgels, Katherine" <krichgels@usgs.gov>
> Subject: Re: [EXTERNAL] RE: DEFUSE documents as submitted
>
> My pleasure - your team did an amazing job getting all the information together in a very short time! Best -Tonie
>
> On Wed, Mar 28, 2018 at 12:07 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:
>
> > ..also want to add my thanks for your help getting this together Tonie!

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4474

www.ecohealthalliance.org

@PeterDaszak

@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Luke Hamel [mailto:hamel@ecohealthalliance.org]
Sent: Wednesday, March 28, 2018 1:00 PM
To: Rocke, Tonie
Cc: Peter Daszak; Alison Andre; Rachel Abbott; Richgels, Katherine
Subject: DEFUSE documents as submitted

Hi Tonie,

Peter had asked me to send these files to you. They are the final versions of our DEFUSE proposal, as submitted yesterday.

Attached files include:

- Technical and Management Proposal (Vol. I)
- Executive Summary Slide
- NWHC budget packet
- NWHC budget justification

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

Fwd: [EXTERNAL] DEFUSE documents as submitted

Richgels, Katherine L <krichgels@usgs.gov>

Wed 3/28/2018 1:10 PM

To: Sleeman, Jonathan M <jsleeman@usgs.gov>; Center Director, GS-MWA-NWHC <nwhc_director@usgs.gov>

4 attachments (7 MB)

PREEMPT Volume 1 no ESS HR001118S0017 EcoHealthAlliance DEFUSE.pdf; Executive Slide HR001118S0017 EcoHealthAlliance DEFUSE.pptx; NWHC budget packet HR001118S0017 EcoHealthAlliance DEFUSE.xlsx; NWHC budget Justification HR001118S0017 EcoHealthAlliance DEFUSE.pdf;

FYI Tonie has submitted the PREEMPT grant.

Katie

Sent from my Verizon, Samsung Galaxy smartphone

-------- Original message --------
From: "Rocke, Tonie" <trocke@usgs.gov>
Date: 3/28/18 2:07 PM (GMT-05:00)
To: "Richgels, Katherine" <krichgels@usgs.gov>
Subject: Fwd: [EXTERNAL] DEFUSE documents as submitted

---------- Forwarded message ----------
From: **Luke Hamel** <hamel@ecohealthalliance.org>
Date: Wed, Mar 28, 2018 at 11:59 AM
Subject: [EXTERNAL] DEFUSE documents as submitted
To: "Rocke, Tonie" <trocke@usgs.gov>
Cc: "Dr. Peter Daszak" <daszak@ecohealthalliance.org>, Alison Andre <andre@ecohealthalliance.org>, Rachel Abbott <rabbott@usgs.gov>, "Richgels, Katherine" <krichgels@usgs.gov>

Hi Tonie,

Peter had asked me to send these files to you. They are the final versions of our DEFUSE proposal, as submitted yesterday.

Attached files include:

- Technical and Management Proposal (Vol. I)
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- NWHC budget packet
- NWHC budget justification

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c)) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

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(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

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ARC - aerosols

William B. Karesh (b) (6) @gmail.com>
Fri 2/2/2018 12:34 PM

To: Rocke, Tonie E <trocke@usgs.gov>; Peter Daszak <daszak@ecohealthalliance.org>
Cc: Luke Hamel <hamel@ecohealthalliance.org>

1 attachments (438 KB)
PARC.pdf;

parc
A Xerox Company

3333 Coyote Hill Road
Palo Alto, CA 94304 USA
+1 650 812 4000
engage@parc.com
www.parc.com

Project Overview

- PARC developed a unique spray technology for large area and high throughput aerosol delivery of highly viscous and concentrated fluids. These fluids can include a range of solutions, e.g., bioactive formulations. This technology has a potential application in large area inoculation of animals/humans with bioengineered formulations for pre-emptive reduction of disease transfer.
- PARC has expertise in fluid/aerosol delivery, leveraging the unique spray method that can aerosolize fluids independent of viscosity or bioactive concentration. This technique enables partners in the biological space to deliver bioactive formulations to animal models with improved chance of efficacy/bioavailability. Potential technical challenges to overcome will be systems integration with rapid development/preparation of pre-emptive agents (potentially with on-demand concentration and composition) and in testing the biological response with animal models.
- PARC can have significant involvement in Technical Area 2 of a PRE-EMPT project: development of a scalable aerosol delivery method for wide-scale inoculation of animal models.

Teaming Overview and Objectives

- PARC has worked with both commercial and university partners for applications of this technology.
- PARC has expertise in fluid delivery, droplet generation, and device and systems integration drawing on our long history with developing printing systems (ink-on-paper). PARC will leverage both previous and on-going work and our related IP portfolio on fluid delivery using platform technologies (spray, transdermal delivery) to meet the PRE-EMPT program objectives.
- PARC has the institutional assets to develop and fabricate new systems for spraying, as well as the background to help improve spray formulation for uptake in mucosal and other targeted membranes.
- PARC is well-positioned to advance its unique spray technology for the PRE-EMPT program, given its demonstrated scalability and wide applicability across different fluids (ranging from low to very high viscosity and independent of bioactive concentration/loading). PARC is looking for collaborators who will investigate disease transmission across animal species and develop the necessary pre-emptive biologicals to prevent such transmission. These engineered biologicals can then be delivered to animal models using the spray technology with maximum chance for efficacy and bioavailability.

Contact Information

Dr. Jerome Unidad; email: Jerome.Unidad@parc.com; telephone: 650-812-4209

First (rough) draft of the DARPA abstract - Project DEFUSE

Peter Daszak <daszak@ecohealthalliance.org>

Wed 2/7/2018 8:51 PM

To: Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>
Cc: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.
Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4473
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

DARPA – PREEMPT – HR001118S0017

Abstract Submission Requirements:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."

B. Executive Summary Slide:

Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at http://www.fbo.gov.

****See slide template at bottom of document.**

PROJECT DEFUSE

C. Goals and Impact:

Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?
Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with high viral loads due to unique damping of their immune systems, likely as an evolutionary adaptation to flight. We will use this to design strategies to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?
Decide which of following parts to talk about:

Modeling bat suitability
Inventory of caves
Sampling/testing
Reverse engineering, binding assays, mouse expts
Modeling viral risk of evolution and spillover
Modeling inoculation/defusing strategy
Immune modulation
Immune Booster recombinant production
Gain-of-function issue.
Inoculum delivery
Mesocosm expts
Cave expts

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SADS), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like SADS-CoV spilling over from bats into intensive pig farms, and devastating and industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?
Will insert this later after calculating and brainstorming.
46 months

Commented [PD1]: Check on the duration of PREEMPT

D. Technical Plan:
Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.
****Note: "The technical plan should demonstrate a deep understanding of the technical challenges and present a credible (even if risky) plan to achieve the program goal"**
Key Terms/Aspects to Emphasize in Abstract

- IACUC/IRB
    - DARPA wants to know who has experience w/ ACURO IACUC work.
        - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
        - IRB also in place, just has to be modified

Overview

Rationale for the SE Asian SARS-related CoV – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous and roost in dense colonies at a fixed, known location, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs trialed out in people may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group has worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for over 10 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and characterization of their ability to bind with human cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making

Commented [PD2]: I know this is too long. I'll edit later this weekend, but want to keep this text for the full proposal

sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case, which makes validated prevention of spillover challenging. These viruses also show little strain diversity which makes modeling which evolutionary lines will be more high-risk, a challenge. SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified a single cave in Yunnan that harbors every gene from the SARS-CoV in a diversity of SARSr-CoVs within the bat population, making it an ideal evolutionary soup to target for intervention.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas are likely to be far less effective in bats because most bats are long-lived relative to their small size, with long inter-generational periods (2-5 years). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

<u>TA1</u>: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and includes a cave in which we have identified all the genetic components of SARS-CoV distributed across a bat population. Two other caves will act as controls/comparison sites, in that we have not yet identified the high-risk SARSr-CoVs in that cave. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic

distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- and satellite telemetry to identify the home range of each species of bat in the caves, to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

We will build environmental niche models using the data above, and environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARSr-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to assess the feasibility of surveys using pooled fresh fecal and urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Proteins that bind will then be inserted into SARS-CoV backbones, and inoculated into humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV (REF).

The modeling team will use these data to build models of 1) risk of viral

Commented [PD3]: Could add " We will continue monitoring the human population proximal to these caves to assess the rates of viral spillover, and ground-truth which specific CoVs are able to infect people

Commented [PD4]: Ralph, Zhengli. If we win this contract, I do not propose that all of this work will necessarily be conducted by Ralph, but I do want to stress the US side of this proposal so that DARPA are comfortable with our team. Once we get the funds, we can then allocate who does what exact work, and I believe that a lot of these assays can be done in Wuhan as well…

evolution and spillover, and 2) strategies to maximize inoculation strategy.
Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve. Because of our unique collaboration among world-class modelers, and coronavirologists, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-2 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra, Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches, the data above, and other biological and ecological data to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation required for both approaches, what proportion of a population needs to be reached to have effective viral dampening, and whether specific seasons, or locations within a cave would be more effective to treat. We will then model the efficacy of different delivery methods (spray, swab, cave mouth automated delivery, deliver to specific sections of a cave).

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

Our goal is to use two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators to up-regulate their naïve immunity to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will produce, and trial inoculation of live bats to suppress the replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-

regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered

by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We will conduct initial experiments on a lab colony of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting infection experiments on this bat genus …(details and citation if possible). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

Finally, work on a delivery method will be overseen by Dr. Tonie Rocke at the National Wildlife Health Center who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using her captive Jamaican fruitbat colony (*10, 11*), Dr. Rocke will trial out the following strategies for delivery of the molecules, inocula proposed above: 1) aerosolization; 2) transdermally applied nanoparticles; 3) sticky edible spray that bats will groom from each other; 4) automated spray triggered by timers and movement detectors at critical cave entry points.. (Details and ideas please Tonie!). These approaches will then be trialed out on live bats in our three cave sites in Yunnan Province. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods trialed out. A small number of bats will be captured and assayed for viral load after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets

collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.

****Note: While only the proposal requires an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years' experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project.

Team:
Lead Organization: EcoHealth Alliance, New York
PI: Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year
Key Personnel:
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year
TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

Subcontract #1: University of North Carolina Medical School
Organizational Lead: Prof. Ralph Baric Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year

Subcontract #2: USGS National Wildlife Health Center
Organizational Lead: Tonie Rocke Ph.D., 2 months/year, no salary requested
TBD Research Technician, 9 months/year

Subcontract #3: Duke NUS, Singapore
Organizational Lead: Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

Subcontract #4: Wuhan Institute of Virology, China
Organizational Lead: Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography

Links to relevant papers, reports, or resumes of key performers.

Do not include more than two resumes as part of the abstract.

****Resumes count against the abstract page limit.**

Commented [PD5]: I'm planning to use my resume and Ralph's. Linfa/Zhengli, I realize your resumes are also very impressive, but I am trying to downplay the non-US focus of this proposal so that DARPA doesn't see this as a negative.

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission. His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

1) 5.1.1. Overall Scientific and Technical Merit

The proposed technical approach is innovative, feasible, achievable, and complete. Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies

major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

2) 5.1.2. Potential Contribution and Relevance to the DARPA Mission

The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In
addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

3) 5.1.3. Cost Realism

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.**

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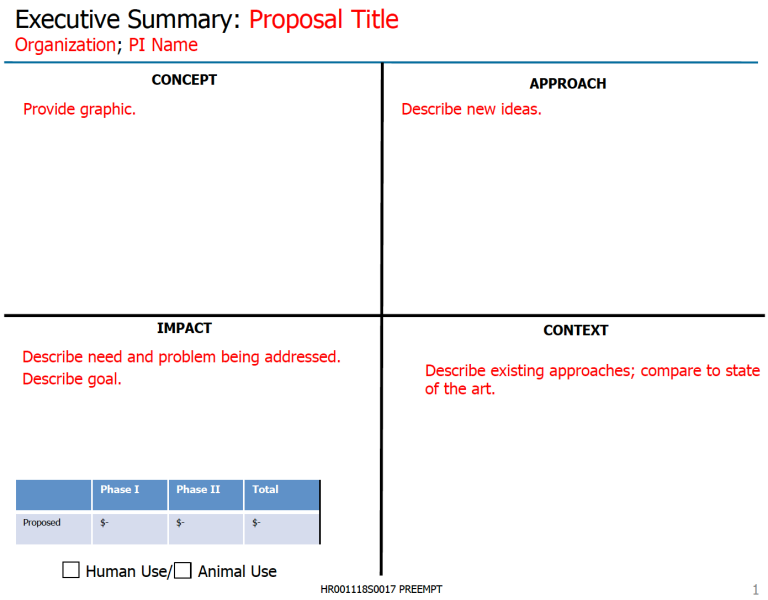

Attachment 1: Executive Summary Slide template

Citations

1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).
7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).

8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

RE: First (rough) draft of the DARPA abstract - Project DEFUSE

Wang Linfa <linfa.wang@duke-nus.edu.sg>

Thu 2/8/2018 6:24 AM

To: Peter Daszak <daszak@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>
Cc: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>

See my brief notes/edits in the attached.

I am working on a large grant here in SG and won't be able to spend too much time until next week.

LF

Linfa (Lin-Fa) WANG, PhD FTSE
Professor & Director
Programme in Emerging Infectious Disease
Duke-NUS Medical School,
8 College Road, Singapore 169857
Tel: +65 6516 8397

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Thursday, 8 February, 2018 10:51 AM
To: Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Rocke, Tonie
Cc: Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Anna Willoughby; Hongying Li
Subject: First (rough) draft of the DARPA abstract - Project DEFUSE
Importance: High

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.
Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4473
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

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intended recipient, please delete it and notify us immediately; you should not copy or use it for any purpose, nor disclose its contents to any other person. Thank you.

DARPA – PREEMPT – HR001118S0017

Abstract Submission Requirements:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."

B. Executive Summary Slide:

Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at http://www.fbo.gov.

****See slide template at bottom of document.**

PROJECT DEFUSE

C. Goals and Impact:

Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with the host without causing diseases due to unique damping of certain pathways in their immune systems, likely as an evolutionary adaptation to flight. We will use this new finding to design strategies to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (broad immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (targeted immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?

Decide which of following parts to talk about:

Commented [L1]: My understanding is that the project will have two parts: A) better risk assessment and modeling and B) risk defusing.

Do we need to say anything about A here?!

Deleted: high viral loads

Deleted: their

Commented [L2]: This will become important late: while we are specifically targeting SARS-realted CoVS, this strategy will be applicable to ALL bat-borne viruses in future

Modeling bat suitability
Inventory of caves
Sampling/testing
Reverse engineering, binding assays, mouse expts
Modeling viral risk of evolution and spillover
Modeling inoculation/defusing strategy
Immune modulation
Immune Booster recombinant production
Gain-of-function issue.
Inoculum delivery
Mesocosm expts
Cave expts

Commented [L3]: I have highlighted the ones which are most challenging and novel for this proposal

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5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SADS), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like SADS-CoV spilling over from bats into intensive pig farms, and devastating and industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?
Will insert this later after calculating and brainstorming.
46 months

Commented [PD4]: Check on the duration of PREEMPT

D. Technical Plan:
Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.
****Note: "The technical plan should demonstrate a deep understanding of the technical challenges and present a credible (<u>even if risky</u>) plan to achieve the program goal"**
<u>Key Terms/Aspects to Emphasize in Abstract</u>

- IACUC/IRB
    - DARPA wants to know who has experience w/ ACURO IACUC work.
        - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
        - IRB also in place, just has to be modified

Overview

Rationale for the SE Asian SARS-related CoV – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous and roost in dense colonies at a fixed, known location, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs trialed out in people may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group has worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for over 10 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and characterization of their ability to bind with human cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making

Commented [PD5]: I know this is too long. I'll edit later this weekend, but want to keep this text for the full proposal

sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case, which makes validated prevention of spillover challenging. These viruses also show little strain diversity which makes modeling which evolutionary lines will be more high-risk, a challenge. SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified a single cave in Yunnan that harbors every gene from the SARS-CoV in a diversity of SARSr-CoVs within the bat population, making it an ideal evolutionary soup to target for intervention.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas are likely to be far less effective in bats because most bats are long-lived relative to their small size, with long inter-generational periods (2-5 years). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

Commented [L6]: We need to provide background info about bat immunity and the track record of this group in the field

Commented [L7]: Peng: I am working on an important grant here in Singapore. Can you add a few points here? Thanks

<u>TA1</u>: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and includes a cave in which we have identified all the genetic components of SARS-CoV distributed across a bat population. Two other caves will act as controls/comparison sites, in that we have not yet identified the high-risk SARSr-CoVs in that cave. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic

distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- and satellite telemetry to identify the home range of each species of bat in the caves, to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

We will build environmental niche models using the data above, and environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARSr-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to assess the feasibility of surveys using pooled fresh fecal and urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Proteins that bind will then be inserted into SARS-CoV backbones, and inoculated into humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV (REF).

The modeling team will use these data to build models of 1) risk of viral

Commented [PD8]: Could add " We will continue monitoring the human population proximal to these caves to assess the rates of viral spillover, and ground-truth which specific CoVs are able to infect people

Commented [PD9]: Ralph, Zhengli. If we win this contract, I do not propose that all of this work will necessarily be conducted by Ralph, but I do want to stress the US side of this proposal so that DARPA are comfortable with our team. Once we get the funds, we can then allocate who does what exact work, and I believe that a lot of these assays can be done in Wuhan as well…

evolution and spillover, and 2) strategies to maximize inoculation strategy.
Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve. Because of our unique collaboration among world-class modelers, and coronavirologists, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-2 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra, Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches, the data above, and other biological and ecological data to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation required for both approaches, what proportion of a population needs to be reached to have effective viral dampening, and whether specific seasons, or locations within a cave would be more effective to treat. We will then model the efficacy of different delivery methods (spray, swab, cave mouth automated delivery, deliver to specific sections of a cave).

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

Our goal is to use two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators to up-regulate their naïve immunity to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will produce, and trial inoculation of live bats to suppress the replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-

regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered

by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We have found that the immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established a breeding colony of cave nectar bats for experimental use (one of very few experimental bat breeding colonies in the world and the only one in SE Asia!). So our initial proof of concept test can be done in this experimental colony. We will then extend the test to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with bat species from the same genus in the BSL4 facility at the Australian Animal Health Laboratory in Australia (L.Wang, unpublished results). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

Deleted: We will conduct initial experiments on a lab colony of wild-caught

Deleted: on this bat

Deleted: …(details and citation if possible).

Finally, work on a delivery method will be overseen by Dr. Tonie Rocke at the National Wildlife Health Center who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using her captive Jamaican fruitbat colony (*10, 11*), Dr. Rocke will trial out the following strategies for delivery of the molecules, inocula proposed above: 1) aerosolization; 2) transdermally applied nanoparticles; 3) sticky edible spray that bats will groom from each other; 4) automated spray triggered by timers and movement detectors at critical cave entry points.. (Details and ideas please Tonie!). These approaches will then be trialed out on live bats in our three cave sites in

Yunnan Province. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods trialed out. A small number of bats will be captured and assayed for viral load after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.

****Note: While only the proposal requires an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years'

experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project.

Team:
Lead Organization: EcoHealth Alliance, New York
PI: Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year
Key Personnel:
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year
TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

Subcontract #1: University of North Carolina Medical School
Organizational Lead: Prof. Ralph Baric Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year

Subcontract #2: USGS National Wildlife Health Center
Organizational Lead: Tonie Rocke Ph.D., 2 months/year, no salary requested
TBD Research Technician, 9 months/year

Subcontract #3: Duke NUS, Singapore
Organizational Lead: Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

Subcontract #4: Wuhan Institute of Virology, China
Organizational Lead: Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography
Links to relevant papers, reports, or resumes of key performers.
Do not include more than two resumes as part of the abstract.
****Resumes count against the abstract page limit.**

Commented [PD10]: I'm planning to use my resume and Ralph's. Linfa/Zhengli, I realize your resumes are also very impressive, but I am trying to downplay the non-US focus of this proposal so that DARPA doesn't see this as a negative.

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission. His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

1) **5.1.1. Overall Scientific and Technical Merit**

The proposed technical approach is innovative, feasible, achievable, and complete. Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

2) **5.1.2. Potential Contribution and Relevance to the DARPA Mission**

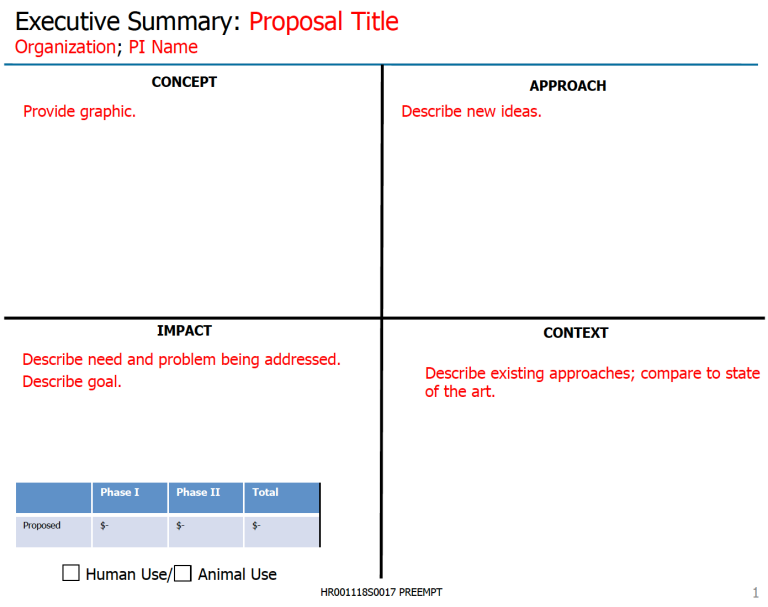
The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In
addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

3) **5.1.3. Cost Realism**

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).
It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to**

offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.

Commented [EA11]: Please note



Attachment 1: Executive Summary Slide template

Citations

1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).

6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).
7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).
8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

RE: First (rough) draft of the DARPA abstract - Project DEFUSE

Baric, Ralph S <rbaric@email.unc.edu>

Thu 2/8/2018 10:22 AM

To: Wang Linfa <linfa.wang@duke-nus.edu.sg>; Peter Daszak <daszak@ecohealthalliance.org>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>
Cc: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>

I have built in my comments atop of Linfa's comments. ralph

From: Wang Linfa [mailto:linfa.wang@duke-nus.edu.sg]
Sent: Thursday, February 8, 2018 7:25 AM
To: Peter Daszak <daszak@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
Cc: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>
Subject: RE: First (rough) draft of the DARPA abstract - Project DEFUSE

See my brief notes/edits in the attached.

I am working on a large grant here in SG and won't be able to spend too much time until next week.

LF

Linfa (Lin-Fa) WANG, PhD FTSE
Professor & Director
Programme in Emerging Infectious Disease
Duke-NUS Medical School,
8 College Road, Singapore 169857
Tel: +65 6516 8397

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Thursday, 8 February, 2018 10:51 AM
To: Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Rocke, Tonie
Cc: Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Anna Willoughby; Hongying Li
Subject: First (rough) draft of the DARPA abstract - Project DEFUSE
Importance: High

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my

hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.
Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4473
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

DARPA – PREEMPT – HR001118S0017

Abstract Submission Requirements:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."

B. Executive Summary Slide:

Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at http://www.fbo.gov.

****See slide template at bottom of document.**

PROJECT DEFUSE

C. Goals and Impact:

Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with the host without causing diseases due to unique damping of certain pathways in their immune systems, likely in part as an evolutionary adaptation to flight. We will use this new finding to design strategies like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (broad immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (targeted immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?

Commented [L1]: My understanding is that the project will have two parts: A) better risk assessment and modeling and B) risk defusing.

Do we need to say anything about A here?!

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Commented [L2]: This will become important late: while we are specifically targeting SARS-realted CoVS, this strategy will be applicable to ALL bat-borne viruses in future

Commented [BRS3]: I thought we were also going to use innate immune antagonists to boost baseline immunity, which should attenuate virus burden in animals?

Isn't this supposed to be a two pronged approach that are complementary, e.g., in that innate immune agonists will also boost immunity to recombinant spike vaccines.

Decide which of following parts to talk about:

Modeling bat suitability

Inventory of caves

Sampling/testing

Reverse engineering, binding assays, mouse expts

Modeling viral risk of evolution and spillover

Modeling inoculation/defusing strategy

Immune modulation

Immune Booster recombinant production

Gain-of-function issue.

Inoculum delivery

Mesocosm expts

Cave expts

5. Who will care and what will the impact be if you are successful?

This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea, Vietnam and Cambodia?). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SARS and related prepandemic zoonotic strains), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like SADS-CoV spilling over from bats into intensive pig farms, and devastating and industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?

Will insert this later after calculating and brainstorming.

46 months

D. Technical Plan:

Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.

****Note: "The technical plan should demonstrate a deep understanding of the technical challenges and present a credible (even if risky) plan to achieve**

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the program goal"

Key Terms/Aspects to Emphasize in Abstract

- IACUC/IRB
  - DARPA wants to know who has experience w/ ACURO IACUC work.
    - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
    - IRB also in place, just has to be modified

Overview

Rationale for the SE Asian SARS-related CoV – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially highly heterogeneous zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous and roost in dense colonies at fixed, known locations, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs evaluated in people and rodents may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group has worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for over 10 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and characterization of their ability to bind and replicate efficiently in primary human lung airway cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have

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evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case, which makes validated prevention of spillover challenging. These viruses also show little strain diversity which makes modeling which evolutionary lines will be more high-risk, a challenge. SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified SARS-like strains in a single cave in Yunnan that harbor every gene found in the human SARS-CoV strains detected during the 2002-2003 epidemic. Within this bat population, an ideal evolutionary soup exists which can produce new human strains by high frequency RNA recombination and presents a perfect target for 21st generation intervention strategies.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas gene drives that are likely to be far less effective in bats because most bats are long-lived relative to their small size, long inter-generational periods (2-5 yrs) and low progeny (~1-2 pups). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and includes a cave in which we have identified all the genetic components of the 2002-2003 epidemic SARS-CoV distributed across a

Commented [BRS9]: These viruses can either be cultured and/or recovered using reverse genetic strategies.

Commented [BRS10]: Filoviruses pretty diverse, although not anywhere near as diverse as cov. Is this a sampling thing or not likely remains unclear?

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Commented [L12]: We need to provide background info about bat immunity and the track record of this group in the field

Commented [L13]: Peng: I am working on an important grant here in Singapore. Can you add a few points here? Thanks

bat population. Two other caves will act as controls/comparison sites, in that we have not yet identified the high-risk SARSr-CoVs in that cave. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- and satellite telemetry to identify the home range of each species of bat in the caves, to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

Commented [BRS14]: Is surveillance in these other caves equally robust over the past 8 yrs?

Commented [PD15]: Could add " We will continue monitoring the human population proximal to these caves to assess the rates of viral spillover, and ground-truth which specific CoVs are able to infect people

We will build environmental niche models using the data above, and environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARSr-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to assess the feasibility of surveys using pooled fresh fecal and urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of

samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Their group have also devised new strategies to culture SARS-like bat coronaviruses, allowing biological characterization of both high risk strains that can replicate in primary human cells and low risk strains that can only replicate in the presence of exogenous enhancers. Viral spike glycoproteins that bind receptor will then be inserted into SARS-CoV backbones, and inoculated into human cells and humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV ((PMC5798318, PMC5567817, PMC5380844, PMC5578707, PMC4801244, PMC4797993), The Baric group has also demonstrated that a nucleoside analogue inhibitor, GS-5734 (Gilead Inc), blocks epidemic, preepidemic and zoonotic SARS-CoV and SARS-like bat coronavirus replication in primary human airway cells and in mice (PMC5567817). Consequently, they will evaluate the ability of this drug to block replication of newly disovered pre-epidemic and zoonotic high risk strains. As the drug has been used to effectively treat Ebola virus infected patients (PMC4967715, PMC5583641) as well and has potent activity against Nipha and Hendra viruses (PMC5338263), an alternative intervention for military personnel is prophylactic treatment treatment prior to deployment into high risk settings.

The modeling team will use these data to build models of 1) risk of viral evolution and spillover, and 2) strategies to maximize inoculation strategy.
Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve. Because of our unique collaboration among world-class modelers, and coronavirologists, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-2 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra, Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches, the data above, and other biological and ecological data to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation required for both approaches, what proportion of a population needs to be reached to have effective viral dampening, and whether specific seasons, or locations within a cave would be more effective to treat. We will then model

Commented [PD16]: Ralph, Zhengli. If we win this contract, I do not propose that all of this work will necessarily be conducted by Ralph, but I do want to stress the US side of this proposal so that DARPA are comfortable with our team. Once we get the funds, we can then allocate who does what exact work, and I believe that a lot of these assays can be done in Wuhan as well…

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Commented [BRS17]: IN the US, these recombinant SARS CoV are studied under BSL3, not BSL2, especially important for those that are able to bind and replicate in primary human cells.
In china, might be growin these virus under bsl2. US reseachers will likely freak out.

the efficacy of different delivery methods (spray, swab, cave mouth automated delivery, deliver to specific sections of a cave).

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

Our goal is to use two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators to up-regulate their naïve immunity to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will produce, and trial inoculation of live bats to suppress the replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Commented [BRS18]: Like what

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Commented [BRS19]: Transient low level Chronic inflammation sounds better

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon

treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We have found that the immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established a breeding colony of cave nectar bats for experimental use (one of very few experimental bat breeding colonies in the

Commented [BRS20]: This could easily take longer than 3 years. Poly ic, IFN or any type of TLR agonist might be more robust. Might want to test in captive bats infected with SARS or select SARS like viruses, like SHC014, which we could provide.

Commented [BRS21]: We have several papers showing importance of TLR3 and TLR4 signaling in control of SARS pathogenesis. PMC4447251, PMC5473747

Commented [BRS22]: Don't attack the other arm of the program. And I disagree that its superior to vaccination, which potentially provides long-term immunity.

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Commented [BRS23]: The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain (PMC5584442). In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles (PMC4058772, PMC5423355, PMC2883479, PMC5578707, PMC3014161). Initially, we will test various delivery vehicles in controlled conditions in bats in a laboratory setting, taking the best candidate forward for testing in the field.
The Baric laboratory has built recombinant S pike glycoproteins harboring structurally defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broad based immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, thereby reducing disease risk in these animals for multiple years (PMC3977350, PMC2588415).

world and the only one in SE Asia!). So our initial proof of concept test can be done in this experimental colony. We will then extend the test to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with bat species from the same genus in the BSL4 facility at the Australian Animal Health Laboratory in Australia (L.Wang, unpublished results). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

Finally, work on a delivery method will be overseen by Dr. Tonie Rocke at the National Wildlife Health Center who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using her captive Jamaican fruitbat colony (*10, 11*), Dr. Rocke will trial out the following strategies for delivery of the molecules, inocula proposed above: 1) aerosolization; 2) transdermally applied nanoparticles; 3) sticky edible spray that bats will groom from each other; 4) automated spray triggered by timers and movement detectors at critical cave entry points.. (Details and ideas please Tonie!). These approaches will then be trialed out on live bats in our three cave sites in Yunnan Province. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods trialed out. A small number of bats will be captured and assayed for viral load after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and

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responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.

****Note: While <u>only the proposal requires</u> an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years' experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project.

Team:
Lead Organization: EcoHealth Alliance, New York
PI: Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year
Key Personnel:
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year

TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

Subcontract #1: University of North Carolina Medical School
Organizational Lead: Prof. Ralph Baric Ph.D., 2 months/year
Dr. Tim Sheahan (6 months/yr)
Dr. Amy Sims (4 months/yr)
Sarah Leist, Postdoctoral fellow (4 months/yr)
Boyd Yount, Research Analyst, 12 months/year
Trevor Scobey, Research Technician, 6 months/yr

Subcontract #2: USGS National Wildlife Health Center
Organizational Lead: Tonie Rocke Ph.D., 2 months/year, no salary requested
TBD Research Technician, 9 months/year

Subcontract #3: Duke NUS, Singapore
Organizational Lead: Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

Subcontract #4: Wuhan Institute of Virology, China
Organizational Lead: Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography
Links to relevant papers, reports, or resumes of key performers.
Do not include more than two resumes as part of the abstract.
****Resumes count against the abstract page limit.**

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering

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Commented [PD24]: I'm planning to use my resume and Ralph's. Linfa/Zhengli, I realize your resumes are also very impressive, but I am trying to downplay the non-US focus of this proposal so that DARPA doesn't see this as a negative.

and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology and Department of Microbiology and Immunology . His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission and pathogenesis. Dr. Baric and his group have developed a platform strategy to access the potential "preepidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (PMC5798318, PMC5567817, PMC5380844, PMC5578707, PMC4801244, PMC4797993). His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

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****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

1) 5.1.1. Overall Scientific and Technical Merit

The proposed technical approach is innovative, feasible, achievable, and complete. Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

2) 5.1.2. Potential Contribution and Relevance to the DARPA Mission

The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

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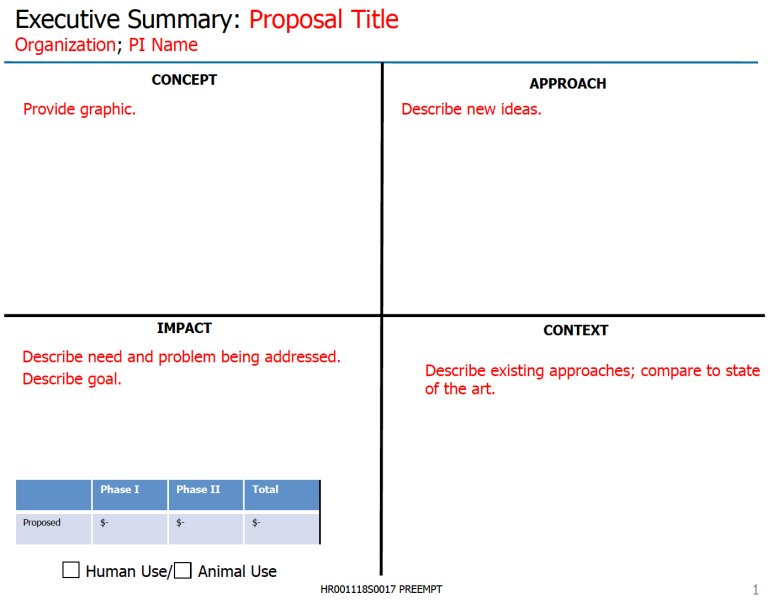
3) 5.1.3. Cost Realism

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.**

Commented [EA25]: Please note



Attachment 1: Executive Summary Slide template

Citations

1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).
7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).

8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

From: Rocke, Tonie <trocke@usgs.gov>
Sent: Thursday, February 8, 2018 10:01 AM
To: Baric, Ralph S
Cc: Wang Linfa; Peter Daszak; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Hongying Li
Subject: Re: First (rough) draft of the DARPA abstract - Project DEFUSE
Attachments: DARPA (PREEMPT) Abstract EcoHealth Alliance DEFUSE 1st Draft-LW180208-rsb-ter.docx

Likewise, I added my comments to Ralph's document. I added some detail, but not too much, so let me know if you want more. Best -Tonie

On Thu, Feb 8, 2018 at 10:22 AM, Baric, Ralph S <rbaric@email.unc.edu> wrote:

> I have built in my comments atop of Linfa's comments. ralph
>
> **From:** Wang Linfa [mailto:linfa.wang@duke-nus.edu.sg]
> **Sent:** Thursday, February 8, 2018 7:25 AM
> **To:** Peter Daszak <daszak@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
> **Cc:** Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>
> **Subject:** RE: First (rough) draft of the DARPA abstract - Project DEFUSE
>
> See my brief notes/edits in the attached.
>
> I am working on a large grant here in SG and won't be able to spend too much time until next week.
>
> LF
>
> ***Linfa (Lin-Fa) WANG, PhD FTSE***

Professor & Director

Programme in Emerging Infectious Disease

Duke-NUS Medical School,

8 College Road, Singapore 169857

Tel: +65 6516 8397

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Thursday, 8 February, 2018 10:51 AM
To: Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Rocke, Tonie
Cc: Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Anna Willoughby; Hongying Li
Subject: First (rough) draft of the DARPA abstract - Project DEFUSE
Importance: High

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.

Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

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New York, NY 10001

Tel. +1 212-380-4473

www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

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Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

DARPA – PREEMPT – HR001118S0017

Abstract Submission Requirements:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."

B. Executive Summary Slide:

Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at http://www.fbo.gov.

****See slide template at bottom of document.**

PROJECT DEFUSE

C. Goals and Impact:

Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with the host without causing diseases due to unique damping of certain pathways in their immune systems, likely in part as an evolutionary adaptation to flight. We will use this new finding to design strategies, like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists, to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (broad immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (targeted immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?

Decide which of following parts to talk about:
Modeling bat suitability
Inventory of caves
Sampling/testing
Reverse engineering, binding assays, mouse expts
Modeling viral risk of evolution and spillover
Modeling inoculation/defusing strategy
Immune modulation
Immune Booster recombinant production
Gain-of-function issue.
Inoculum delivery
Mesocosm expts
Cave expts

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea, Vietnam and Cambodia?). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SARS and related prepandemic zoonotic strains), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like SADS-CoV spilling over from bats into intensive pig farms and devastating the industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?
Will insert this later after calculating and brainstorming.
46 months

D. Technical Plan:
Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.
****Note: "The technical plan should demonstrate a deep understanding of the technical challenges and present a credible (<u>even if risky</u>) plan to achieve**

the program goal"

Key Terms/Aspects to Emphasize in Abstract

- IACUC/IRB
    - DARPA wants to know who has experience w/ ACURO IACUC work.
        - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
        - IRB also in place, just has to be modified

Overview

Rationale for the SE Asian SARS-related CoV – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially highly heterogeneous zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous and roost in dense colonies at fixed, known locations, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs evaluated in people and rodents may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group has worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for over 10 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and characterization of their ability to bind and replicate efficiently in primary human lung airway cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have

evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case- making validated prevention of spillover challenging. These viruses also show little strain diversity ,which also makes it more difficult to model which evolutionary lines are high-risk. Conversely, SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified SARS-like strains in a single cave in Yunnan that harbor every gene found in the human SARS-CoV strains detected during the 2002-2003 epidemic. Within this bat population, an ideal evolutionary soup exists that could produce new human strains by high frequency RNA recombination, and thus, it presents a perfect target for 21st generation intervention strategies.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas gene drives that are likely to be far less effective in bats because most bats are long-lived relative to their small size, have long inter-generational periods (2-5 yrs) and low progeny (~1-2 pups). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and includes a cave in which we have identified all the genetic components of the 2002-2003 epidemic SARS-CoV distributed across a

bat population. Two other caves will act as controls/comparison sites, in that we have not yet identified the high-risk SARSr-CoVs in that cave. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- and satellite telemetry to identify the home range of each species of bat in the caves, to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

We will build environmental niche models using the data above, environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARSr-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to assess the feasibility of surveys using pooled fresh fecal and urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of

samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Their group have also devised new strategies to culture SARS-like bat coronaviruses, allowing biological characterization of both high risk strains that can replicate in primary human cells and low risk strains that can only replicate in the presence of exogenous enhancers. Viral spike glycoproteins that bind receptor will then be inserted into SARS-CoV backbones, and inoculated into human cells and humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV ((PMC5798318, PMC5567817, PMC5380844, PMC5578707, PMC4801244, PMC4797993). The Baric group has also demonstrated that a nucleoside analogue inhibitor, GS-5734 (Gilead Inc), blocks epidemic, preepidemic and zoonotic SARS-CoV and SARS-like bat coronavirus replication in primary human airway cells and in mice (PMC5567817). Consequently, they will evaluate the ability of this drug to block replication of newly disovered pre-epidemic and zoonotic high risk strains. As the drug has been used to effectively treat Ebola virus infected patients (PMC4967715, PMC5583641) as well and has potent activity against Nipha and Hendra viruses (PMC5338263), an alternative intervention for military personnel is prophylactic treatment treatment prior to deployment into high risk settings.

The modeling team will use these data to build models of 1) risk of viral evolution and spillover, and 2) strategies to maximize inoculation strategy.
Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve. Because of our unique collaboration among world-class modelers and coronavirologists, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-2 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra, Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches, the data above, and other biological and ecological data to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation required for both approaches, what proportion of a population needs to be reached to have effective viral dampening, and whether specific seasons, or locations within a cave would be more effective to treat. We will then model

the efficacy of different delivery methods (spray, swab, cave mouth automated delivery, deliver to specific sections of a cave).

<u>TA2:</u> Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

Our goal is to test? two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators designed to up-regulate their naïve immunity and assess their ability to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will conduct inoculation trials with live bats to assess suppression of replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against

filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We have found that the immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established a breeding colony of cave nectar

bats for experimental use (one of very few experimental bat breeding colonies in the world and the only one in SE Asia!). So our initial proof of concept test can be done in this experimental colony. We will then extend the test to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with bat species from the same genus in the BSL4 facility at the Australian Animal Health Laboratory in Australia (L.Wang, unpublished results). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

Finally, work on a delivery method will be overseen by Dr. Tonie Rocke at the US Geological Survey, National Wildlife Health Center, who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using locally acquired insectiverous bats like *Tadarida brasiliensis* or *Eptesicus fuscus* (*10, 11*) as proxies, Dr. Rocke will further develop and assess delivery vehices (mediums) and methods of delivery for the molecules, inocula proposed above, including: 1) transdermally applied nanoparticles; 2) sticky edible gels that bats will groom from themselves and each other; 3) aerosolization via spayers that could be used in cave settings; and 4) automated sprays triggered by timers and movement detectors at critical cave entry points. Simple gels have already been used to vaccinate big brown bats against rabies (11) in a laboratory setting, and hand delivery of these gels containing biomarkers (no vaccine) to vampire bats (*Desmodus rotundus*) in Peru and Mexico have shown they are readily consumed and transferred between bats. Methods to improve uptake (different gels, nanoparticles) and mechanize delivery methods (aerosolization) will be tested first in a laboratry setting, and secondly in local field settings using the biomarker, rhodamine B (which marks hair and whiskers upon consumption) to assess uptake by bats. The most optimal approaches will then be tested on live bats in our three cave sites in Yunnan Province with the most successful immunomodulators developed in TA1?. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods tested. A small number of bats will be captured and assayed for viral load after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental

inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.

****Note: While <u>only the proposal requires</u> an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years' experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project.

Team:

Lead Organization: EcoHealth Alliance, New York

PI: Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year

Key Personnel:
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year
TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

Subcontract #1: University of North Carolina Medical School
Organizational Lead: Prof. Ralph Baric Ph.D., 2 months/year
Dr. Tim Sheahan (6 months/yr)
Dr. Amy Sims (4 months/yr)
Sarah Leist, Postdoctoral fellow (4 months/yr)
Boyd Yount, Research Analyst, 12 months/year
Trevor Scobey, Research Technician, 6 months/yr

Subcontract #2: USGS National Wildlife Health Center
Organizational Lead: Tonie Rocke Ph.D., 2 months/year, no salary requested
TBD Research Technician, 9 months/year

Subcontract #3: Duke NUS, Singapore
Organizational Lead: Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

Subcontract #4: Wuhan Institute of Virology, China
Organizational Lead: Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography

Links to relevant papers, reports, or resumes of key performers.
Do not include more than two resumes as part of the abstract.
****Resumes count against the abstract page limit.**

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology and Department of Microbiology and Immunology . His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission and pathogenesis. Dr. Baric and his group have developed a platform strategy to access the potential "preepidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (PMC5798318, PMC5567817, PMC5380844, PMC5578707, PMC4801244, PMC4797993). His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

1) 5.1.1. Overall Scientific and Technical Merit
The proposed technical approach is innovative, feasible, achievable, and complete.

Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

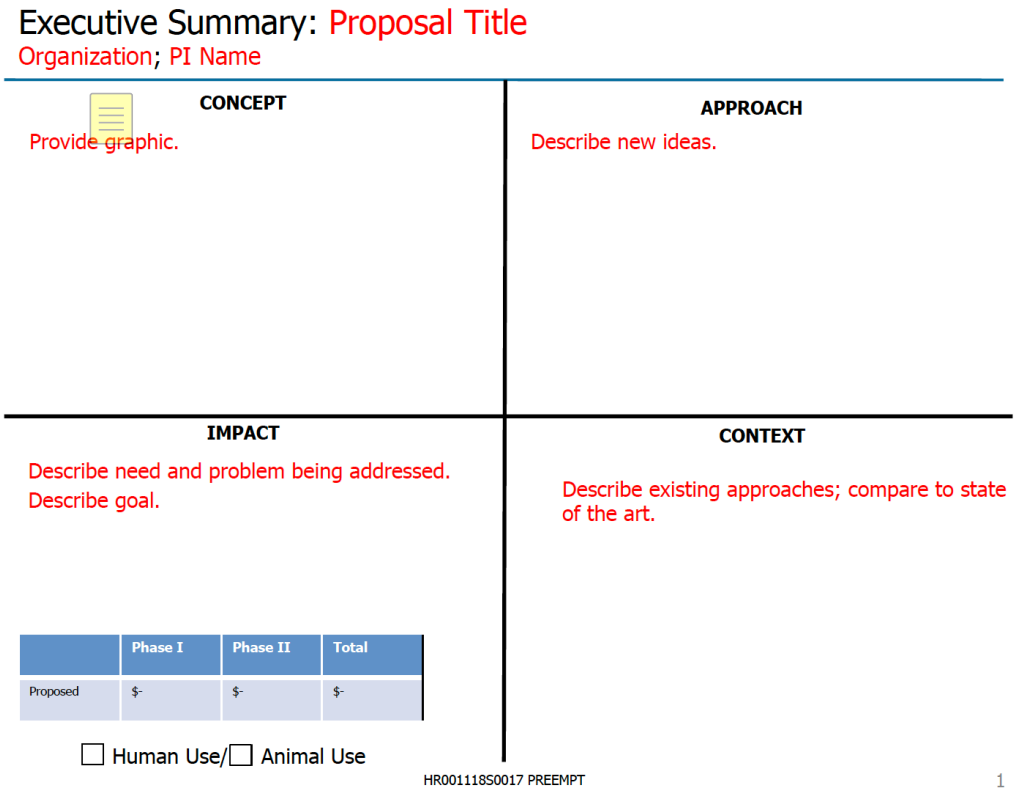
2) 5.1.2. Potential Contribution and Relevance to the DARPA Mission

The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

3) 5.1.3. Cost Realism

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.**



Attachment 1: Executive Summary Slide template

Citations

1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).
7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).

8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

Re: First (rough) draft of the DARPA abstract - Project DEFUSE

Noam Ross <ross@ecohealthalliance.org>

Thu 2/8/2018 11:24 AM

To: Baric, Ralph S <rbaric@email.unc.edu>
Cc: Wang Linfa <linfa.wang@duke-nus.edu.sg>; Peter Daszak <daszak@ecohealthalliance.org>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>; Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>

My changes and comments attached. They primarily
1) Clarify the the modeling components (genotype-phenotype, evolutionary, ecological)
2) Emphasize validation against actual spillover events in the human population

These changes are on Peter's original draft but shouldn't conflict with Linfa and Ralph's.

Noam

On Thu, Feb 8, 2018 at 11:22 AM Baric, Ralph S <rbaric@email.unc.edu> wrote:

> I have built in my comments atop of Linfa's comments. ralph
>
> ---
>
> **From:** Wang Linfa [mailto:linfa.wang@duke-nus.edu.sg]
> **Sent:** Thursday, February 8, 2018 7:25 AM
> **To:** Peter Daszak <daszak@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
> **Cc:** Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>
> **Subject:** RE: First (rough) draft of the DARPA abstract - Project DEFUSE
>
> See my brief notes/edits in the attached.
>
> I am working on a large grant here in SG and won't be able to spend too much time until next week.
>
> LF
>
> ***Linfa (Lin-Fa) WANG, PhD FTSE***

Professor & Director

Programme in Emerging Infectious Disease

Duke-NUS Medical School,

8 College Road, Singapore 169857

Tel: +65 6516 8397

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Thursday, 8 February, 2018 10:51 AM
To: Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Rocke, Tonie
Cc: Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Anna Willoughby; Hongying Li
Subject: First (rough) draft of the DARPA abstract - Project DEFUSE
Importance: High

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.

Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

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New York, NY 10001

Tel. +1 212-380-4473

www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

--

Dr. Noam Ross
Senior Research Scientist

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DARPA – PREEMPT – HR001118S0017

Abstract Submission Requirements:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."

B. Executive Summary Slide:

Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at http://www.fbo.gov.

****See slide template at bottom of document.**

PROJECT DEFUSE

C. Goals and Impact:

Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?
Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with high viral loads due to unique damping of their immune systems, likely as an evolutionary adaptation to flight. We will use this to design strategies to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?
Decide which of following parts to talk about:

Modeling bat suitability
Inventory of caves
Sampling/testing
Reverse engineering, binding assays, mouse expts
Modeling viral risk of evolution and spillover
Modeling inoculation/defusing strategy
Immune modulation
Immune Booster recombinant production
Gain-of-function issue.
Inoculum delivery
Mesocosm expts
Cave expts
Model validation

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SADS), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). In the region directly surrounding our study site, these bat hosts currently roost in unoccupied military bases that may be used by troops at a future time. Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like SADS-CoV spilling over from bats into intensive pig farms, and devastating and industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?
Will insert this later after calculating and brainstorming.
42 months.

Deleted: 46 months

D. Technical Plan:
Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.
****Note: "The technical plan should demonstrate a deep understanding of the**

technical challenges and present a credible (<u>even if risky</u>) plan to achieve the program goal"

<u>Key Terms/Aspects to Emphasize in Abstract</u>

- IACUC/IRB
    - DARPA wants to know who has experience w/ ACURO IACUC work.
        - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
        - IRB also in place, just has to be modified

Overview

Rationale for the SE Asian SARS-related CoV – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous and roost in dense colonies at a fixed, known location, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs trialed out in people may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group has worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for over 10 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and characterization of their ability to bind with human cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have

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evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic. This also gives us the unique ability to validate our models on a significant number of actual spillover events, not only experimental infections; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case, which makes validated prevention of spillover challenging. These viruses also show little strain diversity which makes modeling which evolutionary lines will be more high-risk, a challenge. SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified a single cave in Yunnan that harbors every gene from the SARS-CoV in a diversity of SARSr-CoVs within the bat population, making it an ideal evolutionary soup to target for intervention.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas are likely to be far less effective in bats because most bats are long-lived relative to their small size, with long inter-generational periods (2-5 years). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and the surrounding region and includes a cave in which we have identified all the genetic components of SARS-CoV distributed across a bat population. Two other caves will act as controls/comparison sites, in that we have

not yet identified the high-risk SARSr-CoVs in those caves. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- and satellite telemetry to identify the home range of each species of bat in the caves, to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

We will build environmental niche models using the data above, and environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARSr-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to assess the feasibility of surveys using pooled fresh fecal and urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike

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Commented [PD4]: Could add " We will continue monitoring the human population proximal to these caves to assess the rates of viral spillover, and ground-truth which specific CoVs are able to infect people

Commented [PD5]: Ralph, Zhengli. If we win this contract, I do not propose that all of this work will necessarily be conducted by Ralph, but I do want to stress the US side of this proposal so that DARPA are comfortable with our team. Once we get the funds, we can then allocate who does what exact work, and I believe that a lot of these assays can be done in Wuhan as well…

proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Proteins that bind will then be inserted into SARS-CoV backbones, and inoculated into humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV (REF).

Using both samples from our previous work and new sampling of the human population in the region surrounding our sites, we will determine which viral strains in addition to SARS-CoV have successfully jumped into humans.

The modeling team will use these data to build models of 1) risk of viral evolution and spillover, and 2) strategies to maximize inoculation strategy.

First, based on binding and infection assays in mouse models, we will develop genotype-to-phenotype models to predict viral ability to infect host cells based on genetic traits. Secondly, data on diversity of bat spike proteins, prevalence of recombinant CoVs, and flow of genes within each bat cave via bat movement and migration, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Finally, ecological data, including viral host species and species home ranges will be used to estimate the likelihood of spillover into human populations.

Because of our unique collaboration among world-class modelers, and coronavirologists, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-2 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra, Nipah), which require BSL-4 level facilities for cell culture. In addition, the high frequency of SARSr-CoV spillover events into the human population in this region gives us the allows us to validate models to a degree not possible in systems where spillover events are extremely rare.

We will use stochastic simulation modeling approaches to characterize the dynamics of viral circulation in these bat populations using the data above and other biological and ecological data. Using this model, we will estimate the frequency, efficacy, and population coverage required for our intervention approaches to effectively suppress the viral population. We will determine the seasons, locations within a cave, and different delivery methods (spray, swab, cave mouth automated) that will be most effective. Finally we will determine the time frame the treatment will be effective until re-colonization or evolution will cause a return of a high-risk SARSr-CoV to the population.

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

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Our goal is to use two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators to up-regulate their naïve immunity to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will produce, and trial inoculation of live bats to suppress the replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating

dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We will conduct initial experiments on a lab colony of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting infection experiments on this bat genus …(details and citation if possible). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

Finally, work on a delivery method will be overseen by Dr. Tonie Rocke at the National Wildlife Health Center who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using her captive Jamaican fruitbat colony (*10, 11*), Dr. Rocke will trial out the following strategies for delivery of the molecules, inocula proposed above: 1) aerosolization; 2) transdermally applied nanoparticles; 3) sticky edible spray that bats will groom from each other; 4) automated spray triggered by timers and movement detectors at critical cave entry points.. (Details and ideas please Tonie!). These approaches will then be trialed out on live bats in our three cave sites in Yunnan Province. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods trialed out. A small number of bats will be captured and assayed for viral load after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.

****Note: While <u>only the proposal requires</u> an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years' experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project.

Team:
Lead Organization: EcoHealth Alliance, New York
PI: Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year
Key Personnel:
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year
TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

Subcontract #1: University of North Carolina Medical School
Organizational Lead: Prof. Ralph Baric Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year

Subcontract #2: USGS National Wildlife Health Center
Organizational Lead: Tonie Rocke Ph.D., 2 months/year, no salary requested

TBD Research Technician, 9 months/year

Subcontract #3: Duke NUS, Singapore
Organizational Lead: Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

Subcontract #4: Wuhan Institute of Virology, China
Organizational Lead: Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography
Links to relevant papers, reports, or resumes of key performers.
Do not include more than two resumes as part of the abstract.
****Resumes count against the abstract page limit.**

Commented [PD6]: I'm planning to use my resume and Ralph's. Linfa/Zhengli, I realize your resumes are also very impressive, but I am trying to downplay the non-US focus of this proposal so that DARPA doesn't see this as a negative.

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission. His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

1) 5.1.1. Overall Scientific and Technical Merit

The proposed technical approach is innovative, feasible, achievable, and complete. Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

2) 5.1.2. Potential Contribution and Relevance to the DARPA Mission

The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In
addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

3) 5.1.3. Cost Realism

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed

subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.**

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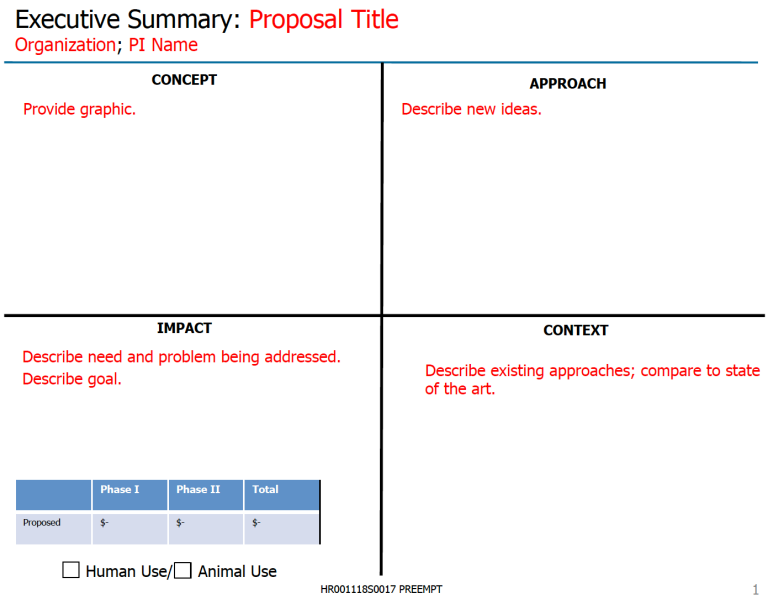

Attachment 1: Executive Summary Slide template

Citations

1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).

3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).
7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).
8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

Re: First (rough) draft of the DARPA abstract - Project DEFUSE

Jon Epstein <epstein@ecohealthalliance.org>

Thu 2/8/2018 1:46 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Baric, Ralph S <rbaric@email.unc.edu>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; Peter Daszak <daszak@ecohealthalliance.org>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>

Attached are my comments.

Cheers,
Jon

On Thu, Feb 8, 2018 at 1:00 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Likewise, I added my comments to Ralph's document. I added some detail, but not too much, so let me know if you want more. Best -Tonie
>
> On Thu, Feb 8, 2018 at 10:22 AM, Baric, Ralph S <rbaric@email.unc.edu> wrote:
>
> > I have built in my comments atop of Linfa's comments. ralph
> >
> > ---
> >
> > **From:** Wang Linfa [mailto:linfa.wang@duke-nus.edu.sg]
> > **Sent:** Thursday, February 8, 2018 7:25 AM
> > **To:** Peter Daszak <daszak@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
> > **Cc:** Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>
> > **Subject:** RE: First (rough) draft of the DARPA abstract - Project DEFUSE
> >
> > See my brief notes/edits in the attached.
> >
> > I am working on a large grant here in SG and won't be able to spend too much time until next week.
> >
> > LF

Linfa (Lin-Fa) WANG, PhD FTSE

Professor & Director

Programme in Emerging Infectious Disease

Duke-NUS Medical School,

8 College Road, Singapore 169857

Tel: +65 6516 8397

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Thursday, 8 February, 2018 10:51 AM
To: Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Rocke, Tonie
Cc: Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Anna Willoughby; Hongying Li
Subject: First (rough) draft of the DARPA abstract - Project DEFUSE
Importance: High

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it

will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.

Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

Peter Daszak

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

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DARPA – PREEMPT – HR001118S0017

Abstract Submission Requirements:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."

B. Executive Summary Slide:

Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at http://www.fbo.gov.

****See slide template at bottom of document.**

PROJECT DEFUSE

C. Goals and Impact:

Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with the host without causing diseases due to unique damping of certain pathways in their immune systems, likely in part as an evolutionary adaptation to flight. We will use this new finding to design strategies like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (broad immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (targeted immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?

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Do we need to say anything about A here?!

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Commented [L2]: This will become important late: while we are specifically targeting SARS-realted CoVS, this strategy will be applicable to ALL bat-borne viruses in future

Commented [BRS3]: I thought we were also going to use innate immune antagonists to boost baseline immunity, which should attenuate virus burden in animals?

Isn't this supposed to be a two pronged approach that are complementary, e.g., in that innate immune agonists will also boost immunity to recombinant spike vaccines.

Decide which of following parts to talk about:
Modeling bat suitability
Inventory of caves
Sampling/testing
Reverse engineering, binding assays, mouse expts
Modeling viral risk of evolution and spillover
Modeling inoculation/defusing strategy
Immune modulation
Immune Booster recombinant production
Gain-of-function issue.
Inoculum delivery
Mesocosm expts
Cave expts

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea, Vietnam and Cambodia?). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SARS and related prepandemic zoonotic strains), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like Severe Acute Diarrheal Syndrome CoV spilling over from bats into intensive pig farms, and devastating and industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?
Will insert this later after calculating and brainstorming.
46 months

D. Technical Plan:
Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.
****Note: "The technical plan should demonstrate a deep understanding of the**

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technical challenges and present a credible (even if risky) plan to achieve the program goal"

Key Terms/Aspects to Emphasize in Abstract

- IACUC/IRB
  - DARPA wants to know who has experience w/ ACURO IACUC work.
    - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
    - IRB also in place, just has to be modified
  - EHA has more than 50 years experience with IACUC (Jon, Billy, Linfa, & Ralph combined, including free-ranging and captive bat species) proposals and currently we have 3 DoD funded projects approved or undergoing ACURO review.

Overview

Rationale for the SE Asian SARS-related CoV – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially highly heterogeneous zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous, common, have a broad geographic range throughout Asia; roost in dense colonies at fixed, known locations, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs evaluated in people and rodents may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group have worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for 15 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and

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characterization of their ability to bind and replicate efficiently in primary human lung airway cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case, which makes validated prevention of spillover challenging. These viruses also show little strain diversity which makes modeling which evolutionary lines will be more high-risk, a challenge. SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified SARS-like strains in a single cave in Yunnan that harbor every gene found in the human SARS-CoV strains detected during the 2002-2003 epidemic. Within this bat population, an ideal evolutionary soup exists which can produce new human strains by high frequency RNA recombination and presents a perfect target for 21st generation intervention strategies.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas gene drives that are likely to be far less effective in bats because most bats are long-lived relative to their small size, long inter-generational periods (2-5 yrs) and low progeny (~1-2 pups per year). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune

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Commented [J10]: However, we may want to highlight that our approach of immune modulation may also reduce the viral load of filoviruses and henipaviruses in cave bat populations. Our work has found Ebola Reston in cave bats in the Philippines (Mineopterus spp.) and henipaviruses and filoviruses have been identified in insectivorous bats in China.

Commented [BRS11]: Filoviruses pretty diverse, although not anywhere near as diverse as cov. Is this a sampling thing or not likely remains unclear?

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Commented [J13]: Yes, correct

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Commented [L15]: Peng: I am working on an important grant here in Singapore. Can you add a few points here? Thanks

boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and includes a cave in which we have identified all the genetic components of the 2002-2003 epidemic SARS-CoV distributed across a bat population. Two other caves will act as controls/comparison sites, in that we have not yet identified the high-risk SARSr-CoVs in these caves. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution and seasonal shedding of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- GPStelemetry to identify the home range of each species of bat in the caves, to identify additional roost sites; to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

We will build ecological niche models using the data above, and environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARS-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often and how far bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to collect fresh fecal and

Commented [BRS16]: Is surveillance in these other caves equally robust over the past 8 yrs?

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Commented [PD17]: Could add " We will continue monitoring the human population proximal to these caves to assess the rates of viral spillover, and ground-truth which specific CoVs are able to infect people

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urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Their group have also devised new strategies to culture SARS-like bat coronaviruses, allowing biological characterization of both high risk strains that can replicate in primary human cells and low risk strains that can only replicate in the presence of exogenous enhancers. Viral spike glycoproteins that bind receptor will then be inserted into SARS-CoV backbones, and inoculated into human cells and humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV ((PMC5798318, PMC5567817, PMC5380844, PMC5578707, PMC4801244, PMC4797993), The Baric group has also demonstrated that a nucleoside analogue inhibitor, GS-5734 (Gilead Inc), blocks epidemic, preepidemic and zoonotic SARS-CoV and SARS-like bat coronavirus replication in primary human airway cells and in mice (PMC5567817). Consequently, they will evaluate the ability of this drug to block replication of newly discovered pre-epidemic and zoonotic high risk strains. As the drug has been used to effectively treat Ebola virus infected patients (PMC4967715, PMC5583641) as well and has potent activity against Nipah and Hendra viruses (PMC5338263), an alternative intervention for military personnel is prophylactic treatment treatment prior to deployment into high risk settings.

The modeling team will use these data to build models of 1) risk of viral evolution and spillover, and 2) strategies to maximize inoculation strategy.
Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve. Because of our unique collaboration among world-class modelers, and virologists with coronavirus expertise, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-3 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra,

Commented [PD18]: Ralph, Zhengli. If we win this contract, I do not propose that all of this work will necessarily be conducted by Ralph, but I do want to stress the US side of this proposal so that DARPA are comfortable with our team. Once we get the funds, we can then allocate who does what exact work, and I believe that a lot of these assays can be done in Wuhan as well…

Commented [J19]: Can we culture any bat coronaviruses? It might be good to broaden this so we can include novel beta CoVs that we may discover which look like they may be transmissible to people

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Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches informed by field and experimental data including the data above and other biological and ecological data, to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation required for both approaches, what proportion of a population needs to be reached to have effective viral dampening, and whether specific seasons, or locations within a cave would be most effective to treat. We will then model the efficacy of different delivery methods (spray, swab, cave mouth automated delivery, deliver to specific sections of a cave).

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

Our goal is to use two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators to up-regulate their naïve immunity to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will produce, and trial inoculation of live bats to suppress the replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe

Commented [BRS20]: IN the US, these recombinant SARS CoV are studied under BSL3, not BSL2, especially important for those that are able to bind and replicate in primary human cells.
In china, might be growin these virus under bsl2. US reseachers will likely freak out.

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Commented [BRS21]: Like what

Commented [J22]: Linfa: Do we know if these findings from Pteropus also apply to Rhinolophus? If not, we should be careful with the assertions we make…
We can make the argument that these two families of bats are more closely related than to other

Commented [BRS23]: Transient low level Chronic inflammation sounds better

inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to be bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

Commented [BRS24]: This could easily take longer than 3 years. Poly ic, IFN or any type of TLR agonist might be more robust. Might want to test in captive bats infected with SARS or select SARS like viruses, like SHC014, which we could provide.

Commented [J25]: If we're proposing experimental work with bats, we should spcify that we'll use SARS-CoV & SADS-CoV host species (Rhinolophus) which can be readily obtained by our Chinese colleagues at WIV

Commented [BRS26]: We have several papers showing importance of TLR3 and TLR4 signaling in control of SARS pathogenesis. PMC4447251, PMC5473747

Commented [BRS27]: Don't attack the other arm of the program. And I disagree that its superior to vaccination, which potentially provides long-term immunity.

Commented [J28]: Agree with Ralph – and this mechanism of delivery would probably be the same for vaccination attempts(intranasal or oral via grooming droplets from fur).

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The Baric laboratory has built recombinant S pike glycoproteins harboring structurally defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broad based immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, thereby reducing disease risk (and transmission risk to people) in these animals for multiple years (PMC3977350, PMC2588415).

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We have found that the immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established a breeding colony of cave nectar bats for experimental use (one of very few experimental bat breeding colonies in the world and the only one in SE Asia!). So our initial proof of concept test can be done in this experimental colony. We will then extend the test to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with bat species from the same genus in the BSL4 facility at the Australian Animal Health Laboratory in Australia (L.Wang, unpublished results). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

Finally, work on a delivery method for immunological countermeasures will be overseen by Dr. Tonie Rocke at the National Wildlife Health Center who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using her captive Jamaican fruitbat colony (*10, 11*), Dr. Rocke will trial out the following strategies for delivery of the molecules, inocula proposed above: 1) aerosolization; 2) transdermally applied nanoparticles; 3) sticky edible spray that bats will groom from each other; 4) automated spray triggered by timers and movement detectors at critical cave entry points.. (Details and ideas please Tonie!). These approaches will then be tested on wild bats in our three cave sites in Yunnan Province. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods trialed out. A small number of bats will be captured and assayed for viral load and immune function after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do

Commented [J30]: Eonycterus and Pteropus are evolutionarily related to Rhinolophus – we may want to have some language asserting our confidence that what we know about bat immunity so far will apply to SARS CoV reservoir species.

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Commented [J31]: We should be clear as to whether we're deploying a vaccine or an immune-modulator that promotes innate immunity. When we mention Tonie's experience with vaccine deployment it looks like that what we're planning.

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Commented [J32]: This probably won't work as bats may move throughout the cave – mixing application techniques. It would be more practical to use a different technique on each cave.

not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.

****Note: While only the proposal requires an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years' experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses, including SARSr-CoVs and MERS-CoV, will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project. The EHA team has extensive experience working with the other team members on previous and current research including Dr. Wang (15+ years); Dr. Shi (15+ years); Dr. Baric (5+ years) and Dr. Rocke (15+ years)

Commented [J33]: [via CCM-NWHC partnership]

Team:

Lead Organization: EcoHealth Alliance, New York
PI: Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year
Key Personnel:
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year
TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

Subcontract #1: University of North Carolina Medical School
Organizational Lead: Prof. Ralph Baric Ph.D., 2 months/year
Dr. Tim Sheahan (6 months/yr)
Dr. Amy Sims (4 months/yr)
Sarah Leist, Postdoctoral fellow (4 months/yr)
Boyd Yount, Research Analyst, 12 months/year
Trevor Scobey, Research Technician, 6 months/yr

Subcontract #2: USGS National Wildlife Health Center
Organizational Lead: Tonie Rocke Ph.D., 2 months/year, no salary requested
TBD Research Technician, 9 months/year

Subcontract #3: Duke NUS, Singapore
Organizational Lead: Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

Subcontract #4: Wuhan Institute of Virology, China
Organizational Lead: Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

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F. If desired, include a brief bibliography

Links to relevant papers, reports, or resumes of key performers.

Do not include more than two resumes as part of the abstract.

****Resumes count against the abstract page limit.**

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology and Department of Microbiology and Immunology . His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission and pathogenesis. Dr. Baric and his group have developed a platform strategy to access the potential "preepidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (PMC5798318, PMC5567817, PMC5380844, PMC5578707, PMC4801244, PMC4797993). His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

Commented [PD34]: I'm planning to use my resume and Ralph's. Linfa/Zhengli, I realize your resumes are also very impressive, but I am trying to downplay the non-US focus of this proposal so that DARPA doesn't see this as a negative.

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1) 5.1.1. Overall Scientific and Technical Merit

The proposed technical approach is innovative, feasible, achievable, and complete. Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

2) 5.1.2. Potential Contribution and Relevance to the DARPA Mission

The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

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3) 5.1.3. Cost Realism

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior**

personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.

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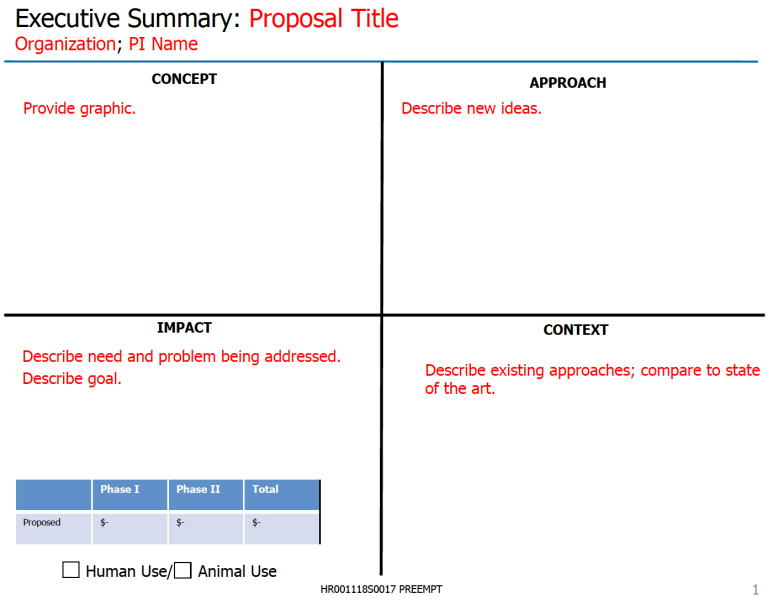

Attachment 1: Executive Summary Slide template

Citations

1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).

7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).
8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

Commented [J36]: Is this reference correct? I couldn't find it online.

From: Rocke, Tonie <trocke@usgs.gov>
Sent: Thursday, February 8, 2018 6:28 AM
To: Richgels, Katherine; Jonathan M Sleeman
Subject: Fwd: First (rough) draft of the DARPA abstract - Project DEFUSE
Attachments: DARPA (PREEMPT) Abstract EcoHealth Alliance DEFUSE 1st Draft.docx

Hi Katie: As I mentioned to you by phone, I have been asked to collaborate on a proposal to DARPA with EcoHealth Alliance. I am forwarding you and Jonathan the first draft I received to keep you in the loop so you know what is going on. There is alot here I need to correct (i.e. we don't have a captive Jamaican fruit bat colony but we are thinking of setting up a vampire bat colony at UW) and I will be working on editing the draft proposal.

Also, I have been asked to collaborate on a second proposal by another group (BU emerging infectious disease unit) for the same RFA that involves morbillivirus and rabies in vampire bats in Latin America, although I haven't seen a draft of that proposal yet.

At this point, I plan to participate in both proposals as there is no restriction in that regard, but let me know soon if you have any questions/concerns. Due date for abstracts is approaching very rapidly - Feb 13. No guarantee of funding of course, but just the fact that we are being asked to collaborate on these proposals should be taken as evidence of the relevance of the NWHC research branch, which seems to be in question lately. -Tonie

---------- Forwarded message ----------
From: **Peter Daszak** <daszak@ecohealthalliance.org>
Date: Wed, Feb 7, 2018 at 8:51 PM
Subject: First (rough) draft of the DARPA abstract - Project DEFUSE
To: "Ralph Baric (rbaric@email.unc.edu)" <rbaric@email.unc.edu>, Wang Linfa <linfa.wang@duke-nus.edu.sg>, "Zhengli Shi (zlshi@wh.iov.cn)" <zlshi@wh.iov.cn>, "William B. Karesh" <karesh@ecohealthalliance.org>, "Rocke, Tonie" <trocke@usgs.gov>
Cc: Luke Hamel <hamel@ecohealthalliance.org>, Jonathon Musser <musser@ecohealthalliance.org>, Anna Willoughby <willoughby@ecohealthalliance.org>, "Kevin Olival, PhD" <olival@ecohealthalliance.org>, Jon Epstein <epstein@ecohealthalliance.org>, Noam Ross <ross@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>, Hongying Li <li@ecohealthalliance.org>

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.

Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4473

www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

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Tonie E. Rocke

USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

DARPA – PREEMPT – HR001118S0017

Abstract Submission Requirements:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."

B. Executive Summary Slide:

Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at http://www.fbo.gov.

****See slide template at bottom of document.**

PROJECT DEFUSE

C. Goals and Impact:

Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?
Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with high viral loads due to unique damping of their immune systems, likely as an evolutionary adaptation to flight. We will use this to design strategies to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?
Decide which of following parts to talk about:

Modeling bat suitability
Inventory of caves
Sampling/testing
Reverse engineering, binding assays, mouse expts
Modeling viral risk of evolution and spillover
Modeling inoculation/defusing strategy
Immune modulation
Immune Booster recombinant production
Gain-of-function issue.
Inoculum delivery
Mesocosm expts
Cave expts

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SADS), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like SADS-CoV spilling over from bats into intensive pig farms, and devastating and industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?
Will insert this later after calculating and brainstorming.
46 months

Commented [PD1]: Check on the duration of PREEMPT

D. Technical Plan:
Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.
****Note: "The technical plan should demonstrate a deep understanding of the technical challenges and present a credible (<u>even if risky</u>) plan to achieve the program goal"**
<u>Key Terms/Aspects to Emphasize in Abstract</u>

- IACUC/IRB
    - DARPA wants to know who has experience w/ ACURO IACUC work.
        - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
        - IRB also in place, just has to be modified

Overview

Rationale for the SE Asian SARS-related CoV – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous and roost in dense colonies at a fixed, known location, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs trialed out in people may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group has worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for over 10 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and characterization of their ability to bind with human cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making

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sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case, which makes validated prevention of spillover challenging. These viruses also show little strain diversity which makes modeling which evolutionary lines will be more high-risk, a challenge. SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified a single cave in Yunnan that harbors every gene from the SARS-CoV in a diversity of SARSr-CoVs within the bat population, making it an ideal evolutionary soup to target for intervention.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas are likely to be far less effective in bats because most bats are long-lived relative to their small size, with long inter-generational periods (2-5 years). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and includes a cave in which we have identified all the genetic components of SARS-CoV distributed across a bat population. Two other caves will act as controls/comparison sites, in that we have not yet identified the high-risk SARSr-CoVs in that cave. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic

distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- and satellite telemetry to identify the home range of each species of bat in the caves, to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

We will build environmental niche models using the data above, and environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARSr-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to assess the feasibility of surveys using pooled fresh fecal and urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Proteins that bind will then be inserted into SARS-CoV backbones, and inoculated into humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV (REF).

The modeling team will use these data to build models of 1) risk of viral

Commented [PD3]: Could add " We will continue monitoring the human population proximal to these caves to assess the rates of viral spillover, and ground-truth which specific CoVs are able to infect people

Commented [PD4]: Ralph, Zhengli. If we win this contract, I do not propose that all of this work will necessarily be conducted by Ralph, but I do want to stress the US side of this proposal so that DARPA are comfortable with our team. Once we get the funds, we can then allocate who does what exact work, and I believe that a lot of these assays can be done in Wuhan as well…

evolution and spillover, and 2) strategies to maximize inoculation strategy.
Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve. Because of our unique collaboration among world-class modelers, and coronavirologists, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-2 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra, Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches, the data above, and other biological and ecological data to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation required for both approaches, what proportion of a population needs to be reached to have effective viral dampening, and whether specific seasons, or locations within a cave would be more effective to treat. We will then model the efficacy of different delivery methods (spray, swab, cave mouth automated delivery, deliver to specific sections of a cave).

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

Our goal is to use two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators to up-regulate their naïve immunity to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will produce, and trial inoculation of live bats to suppress the replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-

regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered

by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We will conduct initial experiments on a lab colony of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting infection experiments on this bat genus …(details and citation if possible). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

Finally, work on a delivery method will be overseen by Dr. Tonie Rocke at the National Wildlife Health Center who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using her captive Jamaican fruitbat colony (*10, 11*), Dr. Rocke will trial out the following strategies for delivery of the molecules, inocula proposed above: 1) aerosolization; 2) transdermally applied nanoparticles; 3) sticky edible spray that bats will groom from each other; 4) automated spray triggered by timers and movement detectors at critical cave entry points.. (Details and ideas please Tonie!). These approaches will then be trialed out on live bats in our three cave sites in Yunnan Province. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods trialed out. A small number of bats will be captured and assayed for viral load after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets

collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.

****Note: While <u>only the proposal requires</u> an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years' experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project.

Team:
Lead Organization: EcoHealth Alliance, New York
PI: Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year
Key Personnel:
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year
TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

Subcontract #1: University of North Carolina Medical School
Organizational Lead: Prof. Ralph Baric Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year

Subcontract #2: USGS National Wildlife Health Center
Organizational Lead: Tonie Rocke Ph.D., 2 months/year, no salary requested
TBD Research Technician, 9 months/year

Subcontract #3: Duke NUS, Singapore
Organizational Lead: Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

Subcontract #4: Wuhan Institute of Virology, China
Organizational Lead: Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography

Links to relevant papers, reports, or resumes of key performers.
Do not include more than two resumes as part of the abstract.
****Resumes count against the abstract page limit.**

Commented [PD5]: I'm planning to use my resume and Ralph's. Linfa/Zhengli, I realize your resumes are also very impressive, but I am trying to downplay the non-US focus of this proposal so that DARPA doesn't see this as a negative.

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission. His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

1) 5.1.1. Overall Scientific and Technical Merit

The proposed technical approach is innovative, feasible, achievable, and complete. Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies

major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

2) 5.1.2. Potential Contribution and Relevance to the DARPA Mission

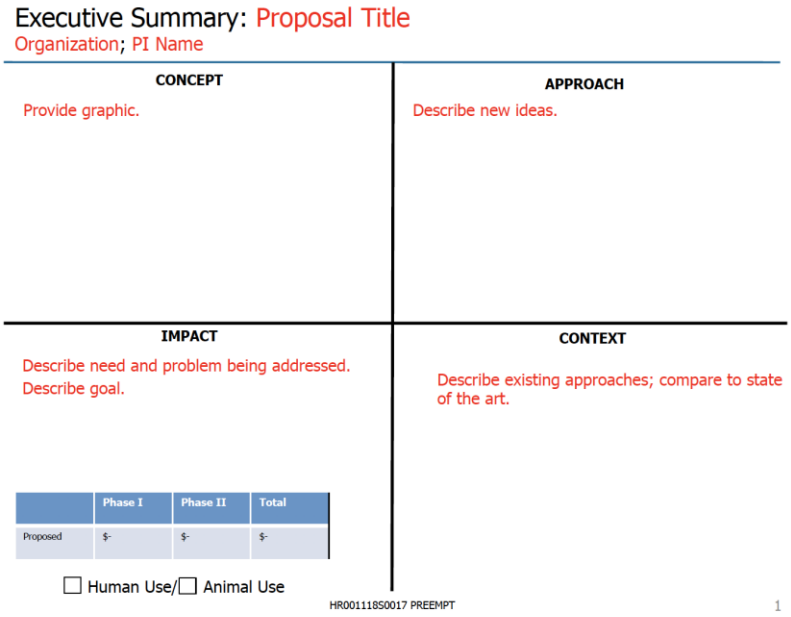
The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In
addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

3) 5.1.3. Cost Realism

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.**

Commented [EA6]: Please note



Attachment 1: Executive Summary Slide template

Citations

1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).
7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).

8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

Re: First (rough) draft of the DARPA abstract - Project DEFUSE

Sleeman, Jonathan M <jsleeman@usgs.gov>

Fri 2/9/2018 5:42 AM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Richgels, Katherine L <krichgels@usgs.gov>

Hi Tonie,

If Katie concurs I am supportive. Peter always has crazy ideas. I would also like to talk to you about the vampire bat colony when we are both in the office.

By the way, no one at the NWHC questions the value of the Research Branch. In fact, it is quite the contrary,

Best wishes,

Jonathan

On Thu, Feb 8, 2018 at 8:27 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Katie: As I mentioned to you by phone, I have been asked to collaborate on a proposal to DARPA with EcoHealth Alliance. I am forwarding you and Jonathan the first draft I received to keep you in the loop so you know what is going on. There is alot here I need to correct (i.e. we don't have a captive Jamaican fruit bat colony but we are thinking of setting up a vampire bat colony at UW) and I will be working on editing the draft proposal.
>
> Also, I have been asked to collaborate on a second proposal by another group (BU emerging infectious disease unit) for the same RFA that involves morbillivirus and rabies in vampire bats in Latin America, although I haven't seen a draft of that proposal yet.
>
> At this point, I plan to participate in both proposals as there is no restriction in that regard, but let me know soon if you have any questions/concerns. Due date for abstracts is approaching very rapidly - Feb 13. No guarantee of funding of course, but just the fact that we are being asked to collaborate on these proposals should be taken as

evidence of the relevance of the NWHC research branch, which seems to be in question lately. -Tonie

---------- Forwarded message ----------
From: **Peter Daszak** <daszak@ecohealthalliance.org>
Date: Wed, Feb 7, 2018 at 8:51 PM
Subject: First (rough) draft of the DARPA abstract - Project DEFUSE
To: "Ralph Baric (rbaric@email.unc.edu)" <rbaric@email.unc.edu>, Wang Linfa <linfa.wang@duke-nus.edu.sg>, "Zhengli Shi (zlshi@wh.iov.cn)" <zlshi@wh.iov.cn>, "William B. Karesh" <karesh@ecohealthalliance.org>, "Rocke, Tonie" <trocke@usgs.gov>
Cc: Luke Hamel <hamel@ecohealthalliance.org>, Jonathon Musser <musser@ecohealthalliance.org>, Anna Willoughby <willoughby@ecohealthalliance.org>, "Kevin Olival, PhD" <olival@ecohealthalliance.org>, Jon Epstein <epstein@ecohealthalliance.org>, Noam Ross <ross@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>, Hongying Li <li@ecohealthalliance.org>

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.

Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4473

www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

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Jonathan Sleeman, MA, VetMB, Dipl. ACZM, Dipl. ECZM, MRCVS
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The USGS National Wildlife Health Center's mission is to safeguard wildlife and ecosystem health through dynamic partnerships and exceptional science

OIE Collaborating Centre for Research, Diagnosis and Surveillance of Wildlife Pathogens

Re: First (rough) draft of the DARPA abstract - Project DEFUSE

Hongying Li <li@ecohealthalliance.org>

Fri 2/9/2018 8:55 PM

To: Jon Epstein <epstein@ecohealthalliance.org>
Cc: Rocke, Tonie E <trocke@usgs.gov>; Baric, Ralph S <rbaric@email.unc.edu>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; Peter Daszak <daszak@ecohealthalliance.org>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; 周鹏 <peng.zhou@wh.iov.cn>

Dear All,

Attached please find comments from Zhengli and Peng. Peng is also helping address the comments from Linfa regarding bat immunity.

Best,
Hongying

Hongying Li, MPH 李泓萤
China Program Coordinator

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.917.573.2178 (U.S. mobile)
86.130.4112.0837 (China mobile)
Hongying Li (Skype)
756614210 (WeChat)

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Thu, Feb 8, 2018 at 2:46 PM, Jon Epstein <epstein@ecohealthalliance.org> wrote:

> Attached are my comments.
>
> Cheers,
> Jon
>
> On Thu, Feb 8, 2018 at 1:00 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

Likewise, I added my comments to Ralph's document. I added some detail, but not too much, so let me know if you want more. Best -Tonie

On Thu, Feb 8, 2018 at 10:22 AM, Baric, Ralph S <rbaric@email.unc.edu> wrote:

> I have built in my comments atop of Linfa's comments. ralph
>
> ---
>
> **From:** Wang Linfa [mailto:linfa.wang@duke-nus.edu.sg]
> **Sent:** Thursday, February 8, 2018 7:25 AM
> **To:** Peter Daszak <daszak@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
> **Cc:** Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>
> **Subject:** RE: First (rough) draft of the DARPA abstract - Project DEFUSE
>
> See my brief notes/edits in the attached.
>
> I am working on a large grant here in SG and won't be able to spend too much time until next week.
>
> LF
>
> ***Linfa (Lin-Fa) WANG, PhD FTSE***
>
> **Professor & Director**
>
> **Programme in Emerging Infectious Disease**
>
> **Duke-NUS Medical School,**
>
> **8 College Road, Singapore 169857**
>
> **Tel: +65 6516 8397**

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Thursday, 8 February, 2018 10:51 AM
To: Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Rocke, Tonie
Cc: Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Anna Willoughby; Hongying Li
Subject: First (rough) draft of the DARPA abstract - Project DEFUSE
Importance: High

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.

Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

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www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

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Jonathan H. Epstein DVM, MPH, PhD

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(b) (6) (mobile)

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EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

DARPA – PREEMPT – HR001118S0017

Abstract Submission Requirements:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."

B. Executive Summary Slide:

Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at http://www.fbo.gov.

****See slide template at bottom of document.**

PROJECT DEFUSE

C. Goals and Impact:

Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?
Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to coexist and spillover viruses due to unique features of their immune systems, likely as an evolutionary adaptation to flight. We will use this to design strategies to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?
Decide which of following parts to talk about:

Commented [p1]: so far all evidences point out that bat WON"T generate high Ab titer, whether under naturally or experimental infection. This is very unique compared to other mammals. We hypothesis this is because of a dampened bat antibody response. Thus I suggest a pre-exposure using immune boosting to bats, and a post-exposure using spike to HUMAN!

Deleted: live with high viral loads

Deleted: damping

Commented [p2]: same comment as above

Commented [p3]: sampling and testing might be challenging as we haven't done a thorough analysis in a certain region. We probably need to sequencing full genome, which is costly as well. I guess we mention the issue that need more money but not revolutionary technique…

Modeling bat suitability

Inventory of caves

Sampling/testing

Reverse engineering, binding assays, mouse expts

Modeling viral risk of evolution and spillover

Modeling inoculation/defusing strategy

Immune modulation

Immune Booster recombinant production

Gain-of-function issue.

Inoculum delivery

Mesocosm expts

Cave expts

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5. Who will care and what will the impact be if you are successful?

This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SADS), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like SADS-CoV spilling over from bats into intensive pig farms, and devastating and industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?

Will insert this later after calculating and brainstorming.

46 months

Commented [PD4]: Check on the duration of PREEMPT

D. Technical Plan:

Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.

****Note: "The technical plan should demonstrate a deep understanding of the technical challenges and present a credible (even if risky) plan to achieve the program goal"**

Key Terms/Aspects to Emphasize in Abstract

- IACUC/IRB
    - DARPA wants to know who has experience w/ ACURO IACUC work.
        - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
        - IRB also in place, just has to be modified

Overview

Rationale for the SE Asian SARS-related CoV – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous and roost in dense colonies at a fixed, known location, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs trialed out in people may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group has worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for over 10 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and characterization of their ability to bind with human cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making

Commented [PD5]: I know this is too long. I'll edit later this weekend, but want to keep this text for the full proposal

sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case, which makes validated prevention of spillover challenging. These viruses also show little strain diversity which makes modeling which evolutionary lines will be more high-risk, a challenge. SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified a single cave in Yunnan that harbors every gene from the SARS-CoV in a diversity of SARSr-CoVs within the bat population, making it an ideal evolutionary soup to target for intervention.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas are likely to be far less effective in bats because most bats are long-lived relative to their small size, with long inter-generational periods (2-5 years). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible (same to rodents and primates). Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and includes a cave in which we have identified all the genetic components of SARS-CoV distributed across a bat population . Two other caves will act as controls/comparison sites, in that we have not yet identified the high-risk SARSr-CoVs in that cave. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic

Commented [p6]: this contradict to previous comments that our model can apply to EBOV bats

Commented [p7]: caution that too frequent recombination can also make modeling difficult

Commented [D8]: Ge et al., Nature, 2013; Yang et al., Journal of Virology; Hu et al., PLoS pathogens, 2017

distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- and satellite telemetry to identify the home range of each species of bat in the caves, to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

We will build environmental niche models using the data above, and environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARSr-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to assess the feasibility of surveys using pooled fresh fecal and urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Proteins that bind will then be inserted into SARS-CoV backbones, and inoculated into humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV (REF).

The modeling team will use these data to build models of 1) risk of viral

Commented [PD9]: Could add " We will continue monitoring the human population proximal to these caves to assess the rates of viral spillover, and ground-truth which specific CoVs are able to infect people

Commented [D10]: Ge et al., Nature, 2013; Yang et al., Journal of Virology; Hu et al., PLoS pathogens, 2017

Commented [D11]: Bat serum samples will be tested for antibody (particularly neutralization antibody) against the SARSr-CoV to evaluate the prevalence and lifetime of antibody in bats. Human samples will be collected from people living around cave and tested for SARSr-CoV infection (Wang et al., Virologica Sinica, 2018).

Commented [PD12]: Ralph, Zhengli. If we win this contract, I do not propose that all of this work will necessarily be conducted by Ralph, but I do want to stress the US side of this proposal so that DARPA are comfortable with our team. Once we get the funds, we can then allocate who does what exact work, and I believe that a lot of these assays can be done in Wuhan as well…

evolution and spillover, and 2) strategies to maximize inoculation strategy.
Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve. Because of our unique collaboration among world-class modelers, and coronavirologists, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-2 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra, Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches, the data above, and other biological and ecological data to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation required for both approaches, what proportion of a population needs to be reached to have effective viral dampening, and whether specific seasons, or locations within a cave would be more effective to treat. We will then model the efficacy of different delivery methods (spray, swab, cave mouth automated delivery, deliver to specific sections of a cave).

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

Our goal is to use two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators to up-regulate their naïve immunity to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will produce, and trial inoculation of live bats to suppress the replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-

regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). Thus, bat interferon would be our first choice. Firstly, we will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). Secondly, we will also attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. Thirdly, We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. Fourthly, We will also attempt to boost bat IFN by activating functional bat IFN production pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway and assay for reduced viral loads. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). Lastly, we believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Commented [p13]: Sorry Peter I rearranged this part in a logical order: interferon first, then modulate unique bat interferon pathways, then modulate usual interferon pathways. I tried not to put polyI:C first, as which is not bat unique.

Moved (insertion) [1]

Deleted: Secondly, bat r

Moved (insertion) [2]

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Deleted: gene.

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Deleted: We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN product ... [1]

Moved up [1]: generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available,

Moved up [2]: We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to

Moved up [3]: We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7

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Commented [p14]: same comment as above, I think it should be a backup post-exposure to human but not to bats

Commented [石15]: why with non-bat coronavirus?

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We will conduct initial experiments on a lab colony of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting infection experiments on this bat genus …(details and citation if possible). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

Finally, work on a delivery method will be overseen by Dr. Tonie Rocke at the National Wildlife Health Center who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using her captive Jamaican fruitbat colony (*10, 11*), Dr. Rocke will trial out the following strategies for delivery of the molecules, inocula proposed above: 1) aerosolization; 2) transdermally applied nanoparticles; 3) sticky edible spray that bats will groom from each other; 4) automated spray triggered by timers and movement detectors at critical cave entry points.. (Details and ideas please Tonie!). These approaches will then be trialed out on live bats in our three cave sites in Yunnan Province. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods trialed out. A small number of bats will be captured and assayed for viral load after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission

for these experimental inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.

****Note: While only the proposal requires an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years' experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project.

Team:
Lead Organization: EcoHealth Alliance, New York

PI: Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year
Key Personnel:
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year
TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

Subcontract #1: University of North Carolina Medical School
Organizational Lead: Prof. Ralph Baric Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year

Subcontract #2: USGS National Wildlife Health Center
Organizational Lead: Tonie Rocke Ph.D., 2 months/year, no salary requested
TBD Research Technician, 9 months/year

Subcontract #3: Duke NUS, Singapore
Organizational Lead: Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

Subcontract #4: Wuhan Institute of Virology, China
Organizational Lead: Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography
Links to relevant papers, reports, or resumes of key performers.
Do not include more than two resumes as part of the abstract.

Commented [PD16]: I'm planning to use my resume and Ralph's. Linfa/Zhengli, I realize your resumes are also very impressive, but I am trying to downplay the non-US focus of this proposal so that DARPA doesn't see this as a negative.

****Resumes count against the abstract page limit.**

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission. His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

1) 5.1.1. Overall Scientific and Technical Merit

The proposed technical approach is innovative, feasible, achievable, and complete. Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and

biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

2) 5.1.2. Potential Contribution and Relevance to the DARPA Mission

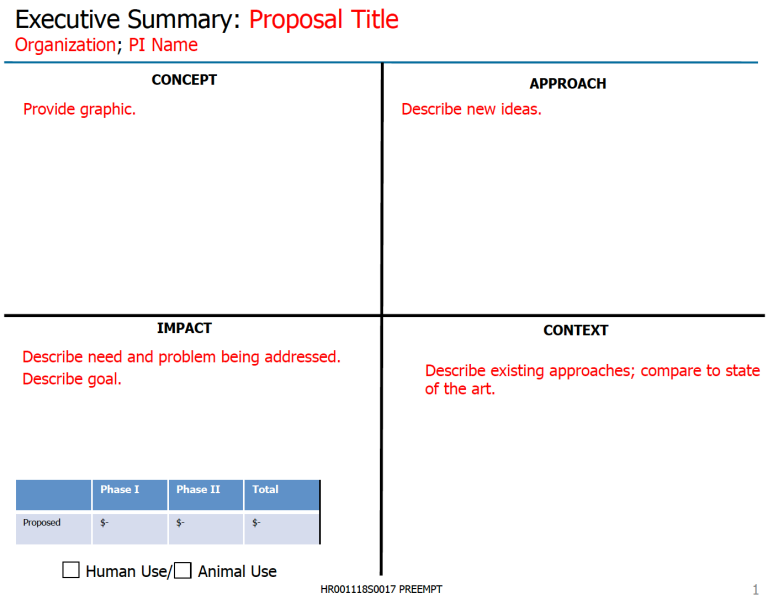
The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In
addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

3) 5.1.3. Cost Realism

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.**

Commented [EA17]: Please note



Attachment 1: Executive Summary Slide template

Citations

1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
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4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).
7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).

8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

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Final draft DARPA abstract

Peter Daszak <daszak@ecohealthalliance.org>

Mon 2/12/2018 11:23 PM

To: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; wang linfa <linfa.wang@duke-nus.edu.sg>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

Luke, attached is the DARPA abstract.

First thing tomorrow, can you go through the references, and create links to the papers on NCBI. Just turn each reference citation: "(*1*)" for example, into a live link to the paper on NCBI. We only have 2 spare lines, so no room to turn each of these into a PMC numbered ref, as Ralph did for his, but please make sure the citation in parentheses is blue, so it's clear it's a live link on the final pdf.

Ralph, Lina, Peng, Zhengli, Tonie – please give this a quick read to make sure I've not said anything completely wrong. I've had to reduce the text a lot to hit the page limit, but I still think it's a great proposal.

I'll finish off the exec summary slide and <500 wd abstract now.

Cheers,

Peter

Peter Daszak
President

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

DARPA – PREEMPT – HR001118S0017- PROJECT DEFUSE

C. Goals and Impact:

1. What is the proposed work attempting to accomplish or do?

We will **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses**. We envisage a scenario whereby the US warfighter is deployed to a security hotspot in SE Asia. As planners choose sites for the mission, they will use an app we will design based on machine-learning models of the ecological and evolutionary potential of bat viruses to spillover. This will allow rapid assessment of the background risk of a site harboring dangerous zoonotic viruses. If there is no alternative site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release **broadscale immune boosting molecules** and chimeric polyvalent spike protein **targeted immune priming inocula** to upregulate the naturally damped innate immune response of bats, and lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

There is no available current technology to reduce the risk of exposure to novel coronaviruses from bats. Models of bat host capacity to harbor viruses, of ecological and environmental drivers of their emergence, and of the evolutionary potential of different strains to spillover are rudimentary. No vaccines, or therapeutics exist for SARSr-CoVs, and exposure mitigation strategis are non-existent. SARSr-CoVs are endemic in Asian, African (*1*), and European bats (*2*) that roost in caves but forage widely at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARSr-CoVs into people in China, and have isolated strains capable of producing SARS-like illness in humanized mice that doesn't respond to antibody treatment or vaccination. These viruses are a clear-and-present danger to our military, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

Our group leads the world in predictive models of viral emergence. We will build on our hotspots, machine-learning, ecological niche and genotype-phenotype modeling by incorporating unique datasets to validate and refine hotspot risk maps of viral emergene in SE Asia and beyond. Our group has shown that bats coexist with lethal viruses by damping innate immunity pathways, likely as an evolutionary adaptation to flight. We will use this insight to design strategies, like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists, to upregulate bat immunity in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover **(broadscale immune boosting strategy)**. We will complement this by inoculating bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against specific, high-risk viruses **(targeted immune priming strategy)**, especially when their immune response is boosted as above. We will design novel methods to deliver

these inocula remotely to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?
Modeling: Previous models have suffered from a lack of data to validate them. We have access to unique datasets that will allow us to validate our approach, including biodiversity surveys of bat caves across S. China, 10+ years of bat viral testing data in China, and 10 other countries (from NIAID and USAID EPT PREDICT work). Uniquely, we will validate our models of viral evolution/spillover risk using serology (based on LIPS assays) in local populations, who have high (~3%) serology to bat SARSr-CoVs.
Identifying Immune boosting and priming inocula: Some of our approaches are novel and challenging (e.g. using CRISPRi to find the negative regulator for bat interferon production), and others are unproven in bats (e.g. Poly IC). We will begin all immune boosting and priming experiments at the beginning of the project, running them simultaneously and competitively, so that we field trial only the most efficient, cost-effective and scalable approaches.

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. Potential deployment to regions where SARSr-CoVs exist is high – countries include security hotspots in Asia (e.g. Myanmar, Bangladesh, Pakistan, Korea, Vietnam), Africa and Eastern Europe. The ability to decontaminate and defuse these viruses may prevent potentially devastating illness. These technologies could be adapted to hosts of other bat-origin CoVs (e.g. MERS-CoV, SADS-CoV), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV), with benefits to livestock production, food security and global public health.

6. How much will it cost and how long will it take?
Aleksei/Luke to fill out
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D. Technical Plan:
Overview
The SARSr-CoV-bat system, and immune modulation focus: Our group's 15 yrs work on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014 & WIV-1). We have shown they bind and replicate efficiently in primary human lung airway cells and that chimeras with SARSr-CoV spike proteins in a SARS-CoV backbone cause SARS-like illness in humanized mice, with clinical signs that are not reduced by SARS monoclonal therapy or vaccination. We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV (*7-9*). We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover,

and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**. Our work on bat immunology suggests that bats' unique flying ability has led to downregulated innate immune genes, and their ability to coexist with viruses such as SARSr-CoVs, henipa- and filoviruses that are lethal in many other mammals (*3*). We have identified bat-specific constitutively expressed bat interferon, a dampened STING-interferon production pathway (*4, 5*), and have identified a series of other innate immunity factors that are dampened in bats (*6*).

Our bat-CoV system has significant advantages for experiment and intervention. Firstly, these viruses are fecal-orally transmitted within bat populations, so sampling can be achieved from fresh fecal pellet collection. They are BSL-3, not -4, agents, so that experimental manipulation and infection is simpler. They have frequent spillover events, making it possible to validate predictive models of spillover by sampling people. They are diverse, with frequent recombination and different strains exhibiting differential host cell binding and spillover potential. Finally, we have identified SARSr-CoV strains in a single cave in Yunnan that harbor all of the epidemic SARS-CoV genes. This specific bat population harbors an ideal evolutionary soup that could produce new human strains by high frequency RNA recombination, and thus, it presents a perfect target for next generation, technology-forward intervention strategies.

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team led by Drs Daszak, Ross, Olival, EHA, will build ecological niche models of environmental and ecological correlates, and traits of cave bat communities to predict species composition of bat caves across Southern China, South and SE Asia. We will then use a series of datasets we have built to produce host-virus risk models for the region. These include our unique database of bat host-viral relationships (*7*); biological inventory data on all bat caves in Southern China; and modeled species distribution data for all bats. We will parameterize the model with data from three cave sites in Yunnan, China (one with high-risk SARSr-CoVs, two other control/comparison sites), including: radio- and GPS-telemetry to identify home range and additional roost sites for each bat species; inventory of bat population density, distribution and segregation and their daily, weekly and seasonal changes; viral prevalence and individual viral load; shedding of low- and high-risk SARSr-CoV strains among bat species, age classes, genders; and telemetry and mark-recapture data to assess metapopulation structure and inter-cave connectivity. We will test and validate model predictions of a cave's viral spillover potential with data from prior PREDICT sampling in 7 other Asian countries. At the end of Yr 1, we will produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens in a region. **The 'high-risk bats near me' app** will be updated real-time with surveillance data (e.g. field-deployable iphone and android compatible echolocation data) from our project and others, to ground-truth and fine-tune its predictive capacity.

The Wuhan Institute of Virology team will test bat fecal, oral, blood and urogenital samples for SARSr-CoVs. We will correlate viral load data from these samples

with fresh fecal pellets from individuals and from tarps laid on cave floors. We will rapidly move to fecal pellet assays to reduce roost disturbance. SARSr-CoV spike proteins will be sequenced, analyzed phylogenetically, for recombination events, and high-risk viruses (spike proteins close to SARS-CoV) characterized and isolated. The UNC team will reverse engineer spike proteins to conduct binding assays to human ACE2 (the SARS-CoV receptor). They will culture SARS-like bat coronaviruses to distinguish high risk strains that can replicate in primary human cells and low risk strains that require exogenous enhancers. Viral spike glycoproteins that bind receptors will be inserted into SARS-CoV backbones, inoculated into human cells and humanized mice to assess capacity to cause SARS-like disease, and to be blocked by monoclonal therapies, the nucleoside analogue inhibitor GS-5734 (*8*) or vaccines against SARS-CoV (*8-13*).

The EHA modeling team will use these data to **build models of risk of viral evolution and spillover**. These genotype-to-phenotype machine-learning models will predict viral ability to infect host cells based on genetic traits and results of receptor binding and mouse infection assays. Using data on diversity of spike proteins, recombinant CoVs, and flow of genes within each bat cave via bat movement and migration, we will estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Finally, virus-host relationship and bat home range data will be used to estimate spillover potential - extending models well beyond our field sites. We will then **validate model predictions of viral spillover risk** by **1)** conducting spike protein-based binding and cell culture experiments, and **2)** identifying spillover strains in people near our bat cave sites. Our preliminary work on this shows ~3% serology to SARSr-CoVs, using a specific ELISA (*14*). We will design LIPS assays to the specific high- and low- zoonotic-risk SARSr-CoVs identified in this project as we have done previously (*15*). We will use banked and newly collected human sera from these populations to test for presence of antibodies to the high- and low-risk SARSr-CoVs identified by our modeling. We will then **model optimal strategies to maximize inoculation efficacy** for TA2, using machine-learning stochastic simulation modeling informed by field and experimental data to characterize viral circulation dynamics in bats. We will estimate frequency and population coverage required for our intervention approaches to suppress viral spillover. We will determine the seasons, locations within a cave, and delivery methods (spray, swab, or automated cave mouth or drone) that will be most effective. Finally we will determine the time period treatment will be effective for, until re-colonization or evolution leads to return of a high-risk SARSr-CoV.

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

We will evaluate two approaches to defuse SARS-related CoV spillover potential**: 1) Broadscale Immune Boosting:** using the unique immune damping in bats that our group has discovered, we will inoculate live bats with immune modulators like bat interferon designed to up-regulate their naïve immunity and then assess their ability to suppress viral replication and shedding; **2) Targeted Immune Priming:** building on preliminary development of polyvalent chimeric recombinant SARSr-CoV spike proteins, we will

conduct inoculation trials with live bats to assess suppression of replication and shedding of a broad range of dangerous SARS-related CoVs.

Both lines of work will begin in Yr 1 and run parallel. Prof. Linfa Wang (Duke-NUS) will lead the immune boosting work, building on his pioneering work on bat immunity (*3*) which shows that the long-term coexistence of bats and their viruses has led to equilibrium between viral replication and host immunity. This is likely due to down-regulation of their innate immune system as a fitness cost of flight (*3*). The weakened functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may allow bats to maintain an effective, but not over-response to viruses (*4*). A similar finding was observed for bat IFNA, which is less abundant but constitutively expressed without stimulation (*5*). Given high native SARSr-CoV load in bats, we aim to boost bat innate immunity through the IFN pathway, break the host-virus equilibrium to suppress bat SARSr-CoV replication and shedding.

We will trial the following concurrently and competitively for efficiency, cost and scalability: **i)** Universal bat interferon. Aerosol spraying or intranasal inoculation of IFN or other small molecules reduces viral loads in humans, ferrets and mouse models (*16, 17*). Interferon has been used clinically when antiviral drugs are unavailable, e.g. against filoviruses (*18*). Replication of SARSr-CoV is sensitive to interferon treatments, as shown in our previous work (*16*); **ii)** Boosting bat IFN by blocking bat-specific IFN negative regulators. Uniquely, bat IFNA is naturally constitutively expressed but cannot be induced to a high level (*5*), indicating a negative regulatory factor in the bat interferon production pathway. We will use CRISPRi to identify the negative regulator and then screen for compounds targeting this gene; **iii)** Activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7-dependent pathways. Our work showing that mutant bat STING restores antiviral functionality suggests these pathways are important in bat-viral coexistence (*4*). By identifying small molecules to directly activate downstream of STING, we will activate bat interferon and promote viral clearance. A similar strategy will be applied to ssRNA-TLR7-dependent pathways; **iv)** Activating functional bat IFN production pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been demonstrated in a mouse model for SARS-CoV, IAV and HBV (*17, 19*); **v)** Inoculating crude coronavirus fragments to upregulate innate immune responses to specific CoVs – a partial step towards the targeted immune priming work below.

Prof. Ralph Baric (UNC) will lead the immune priming work. He will develop recombinant chimeric spike-proteins (*20*) from our known SARSr-CoVs, and those we characterize during project DEFUSE. The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain (*21*). In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles (*11, 22-25*). We will test these in controlled lab conditions, taking the best candidate forward for testing in the field. We have built recombinant spike glycoproteins harboring structurally defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and

zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broadscale immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, potentially reducing disease risk (and transmission risk to people) in these animals for longer periods (*26, 27*).

The immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established the only experimental breeding colony of cave bats (*Eonycteris spelaea*) in SE Asia. This genus is evolutionarily closely related to *Rhinolophus* spp. (the hosts of SARSr-CoVs), so we have confidence that results will be transferable. Our initial proof-of-concept tests will be in this experimental colony, extended to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with *Rhinolophus* sp. bats in the BSL-4 facility at CSIRO, AAHL (L.Wang, unpublished results).

Finally, work on a delivery method for our immune boosting and priming molecules will be overseen by Dr. Tonie Rocke at the USGS, National Wildlife Health Center who has previously developed animal vaccines through to licensure (*28*). Using locally acquired insectivorous bats (*29, 30*) we will assess delivery vehicles and methods including: 1) transdermally applied nanoparticles; 2) series of sticky edible gels that bats will groom from themselves and each other; 3) aerosolization via sprayers that could be used in cave settings; 4) automated sprays triggered by timers and movement detectors at critical cave entry points; 5) sprays delivered by remote controlled drone. We have already used simple gels to vaccinate bats against rabies in the lab (*29*), and hand delivered these containing biomarkers to vampire bats in Peru and Mexico to show they are readily consumed and transferred among bats. In our bat colony, we will trial delivery vehicles using the biomarker rhodamine B (which marks hair and whiskers upon consumption) to assess uptake. The most optimal approaches will then be tested on wild bats in our three cave sites in Yunnan Province with the most successful immunomodulators from TA2. Fieldwork will be conducted under the auspices of Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance). A small number of bats will be captured and assayed for viral load and immune function after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has had unique access to these sites for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for experimental inoculations from the Provincial Forestry Department. We expect to be successful, as we have worked with the Forestry Department collaboratively for 10 years, with support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife. EHA has a proven track record of rapidly obtaining IACUC and DoD ACURO approval for bat research.

E. Capabilities:

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research organization focused on emerging zoonotic diseases. The PI, Dr. Peter Daszak, has 25+ years' experience managing lab, field and modeling research projects on emerging zoonoses. Dr. Daszak will commit 3 months annually to oversee and

coordinate all project activities, and lead modeling and analytic work for TA1. Dr. Billy Karesh has 40+ years' experience leading zoonotic and wildlife disease projects, and will commit 1 month annually to manage partnership activities and outreach. Dr. Jon Epstein, with 15 years' experience working emerging bat zoonoses will coordinate animal trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project. Support staff include field surveillance teams, modeling analysts, and consultants based in Yunnan Province, China, to oversee field trials. The EHA team has worked extensively with all other collaborators: Prof. Wang (15+ years); Dr. Shi (15+ years); Prof. Baric (5+ years) and Dr. Rocke (15+ years).

Subcontracts: #1 to Prof. Ralph Baric, UNC, to oversee reverse engineering of SARSr-CoVs, BSL-3 humanized mouse experimental infections, design and testing of immune priming inocula based on recombinant spike proteins. Assisted by senior personnel Dr. Tim Sheahan, Dr. Amy Sims, and support staff; **#2** to Prof. Linfa Wang, Duke NUS, to oversee the immune boosting approach, captive bat experiments, and analyze immunological and virological responses to immune boosting inocula; **#3** to Dr. Zhengli Shi, Wuhan Institute of Virology, to conduct PCR testing, viral discovery and isolation from bat samples collected in China, spike protein binding assays, and some humanized mouse work, as well as experimental inoculation of *Rhinolophus* bats. Her team will include Dr. Peng Zhou and support staff; **#4** to Dr. Tonie Rocke, USGS National Wildlife Health Center, to refine delivery mechanisms for both immune boosting and immune priming treatments. With a research technician, Dr. Rocke will use a captive colony of bats at NWHC for initial trials, and oversee cave experiments in China.

F. *Links to published papers, resume of two key performers*

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based research organization focused on emerging zoonotic diseases. His >300 scientific papers include the first global map of EID hotspots (*31, 32*), estimates of unknown viral diversity (*33*), predictive models of virus-host relationships (*7*), and evidence of the bat origin of SARS-CoV (*34, 35*) and other emerging viruses (*36-39*). He is Chair of the NASEM Forum on Microbial Threats, and is a member of the Executive Committee and the EHA institutional lead for the $130 million USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Dept. of Epidemiology and Dept. of Microbiology & Immunology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, cross species transmission and pathogenesis. His group has developed a platform strategy to access the potential "pre-epidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (*8-13*).

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RE: Final draft DARPA 500 wd summary

Peter Daszak <daszak@ecohealthalliance.org>
Tue 2/13/2018 12:03 AM

To: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; wang linfa <linfa.wang@duke-nus.edu.sg>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

It's 497 words now.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
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www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Peter Daszak
Sent: Tuesday, February 13, 2018 12:23 AM
To: Luke Hamel (hamel@ecohealthalliance.org);

Technical Approach: Our goal is to defuse the potential for spillover of novel bat-origin high-zoonotic risk SARS-related coronaviruses in Southeast Asia. **In TA1** we will develop **host-pathogen ecological niche models** to predict the species composition of bat caves across Southeast Asia. We will parameterize this with a full inventory of host and virus distribution at our field sites, three caves in Yunnan Province, China and a series of unique datasets on bat host-viral relationships. By the end of Y1, we will use these to create a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens at any site across Asia. We will intensively sample bats at our field sites to sequence SARSr-CoV spike proteins, reverse engineer them to conduct binding assays, and insert them into SARS-CoV backbones to infect humanized mice to assess capacity to cause SARS-like disease. Our modeling team will use these data to build **machine-learning genotype-phenotype models** of viral evolution and spillover risk. We will uniquely validate these with human serology data through LIPS assays designed to assess which spike proteins allow spillover into people.

In TA2, we will evaluate two approaches to reduce SARSr-CoV shedding in cave bats: (**1) Broadscale Immune Boosting**, in which we will inoculate bats with immune modulators to upregulate their innate immune response and downregulate viral replication; **(2) Targeted Immune Priming**, in which we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance innate immunity against specific, high-risk viruses. We will trial inoculum delivery methods on captive bats including automated aerosolization, transdermal nanoparticle application and edible, adhesive gels. We will use stochastic simulation modeling informed by field and experimental data to characterize viral dynamics in our cave sites, to maximize timing, inoculation protocol, delivery method and efficacy of viral suppression. The most effective delivery method and immune modulating treatments will be trialed in our experimental cave sites in Yunnan Province, with reduction in viral shedding as proof-of-concept.

Management Approach: Members of our collaborative group have worked together on bats and their viruses for over 15 years. The lead organization, EcoHealth Alliance, will oversee all modeling, lab, and fieldwork. EHA staff will develop models to evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for delivery of both immune boosting and immune targeting inocula. Specific work will be subcontracted to the following organizations:

1) Prof. Ralph Baric, UNC, will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades.
2) Prof. Linfa Wang, Duke-NUS, will lead work on immune boosting, building from his groups' pioneering work on bat immunity.
3) Dr. Zhengli Shi, Wuhan Institute of Virology will conduct viral testing on all collected samples, binding assays and some humanized mouse work.
4) Dr. Tonie Rocke, USGS National Wildlife Health Center will develop a delivery method for immunological countermeasures, following from her work on vaccine delivery in wildlife, including bats.

RE: Final DARPA Exec Summary slide

Peter Daszak <daszak@ecohealthalliance.org>

Tue 2/13/2018 12:03 AM

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Hi Luke and Jonathon.

Here's the edited, final summary slide.

Please insert the re-drawn figure, and the budget numbers from Aleksei tomorrow before you submit

NB –there are other things to check on the Abstract, including budget numbers, timeline, the references, and then please do a final check on the compliance with DARPA instructions for all!

Cheers,

Peter

Peter Daszak
President

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From: Peter Daszak
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To: Luke Hamel (hamel@ecohealthalliance.org); Jonathon Musser
Cc: William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); wang linfa; 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura (chmura@ecohealthalliance.org); 'Anna Willoughby (willoughby@ecohealthalliance.org)'; Rocke, Tonie
Subject: Final draft DARPA abstract
Importance: High

Luke, attached is the DARPA abstract.

First thing tomorrow, can you go through the references, and create links to the papers on NCBI. Just turn each reference citation: "(*1*)" for example, into a live link to the paper on NCBI. We only have 2 spare lines, so no room to turn each of these into a PMC numbered ref, as Ralph did for his, but please make sure the citation in parentheses is blue, so it's clear it's a live link on the final pdf.

Ralph, Lina, Peng, Zhengli, Tonie – please give this a quick read to make sure I've not said anything completely wrong. I've had to reduce the text a lot to hit the page limit, but I still think it's a great proposal.

I'll finish off the exec summary slide and <500 wd abstract now.

Cheers,

Peter

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Executive Summary: Proposal Title

EcoHealth Alliance; Dr. Peter Daszak

CONCEPT

Provide graphic.

APPROACH

Describe new ideas.

IMPACT

Describe need and problem being addressed.

Describe goal.

CONTEXT

Describe existing approaches; compare to state of the art.

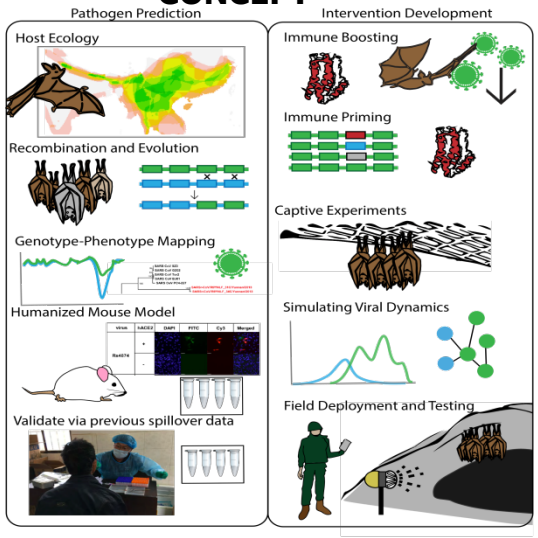
	Phase I	Phase II	Total
Proposed	$-	$-	$-

☒Human Use/ ☒ Animal Use

Executive Summary: DEFUSE

EcoHealth Alliance; Dr. Peter Daszak

CONCEPT



APPROACH

Pathogen Prediction:

- Host-Pathogen Ecology: Develop host-pathogen ecological niche models based on unique bat and viral data, to estimate likelihood of spillover of SARS-related CoVs into human populations. Doing so will enhance predictive ability of models beyond sampling sites in China to cover all Asia.
- Mobile Application: Create 'Reservoirs Near Me' mobile application, to assess background risk of disease spillover for any site across Southeast Asia.
- Binding and Humanized mouse asssays: Utilize team's unique collaboration between world-class modelers and virologists with CoV expertise to conduct spike protein-based binding and humanized mice experiments.
    - Use results to test machine-learning genotype-to-phenotype model predictions of viral spillover risk.
- Genotype-Phenotype Models: Develop models to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Inputs to include: Diversity of bat spike proteins, prevalence of recombinant CoVs, and flow of genes within each bat cave via bat movement and migration.
- Validation with Human Sera: Analyze new and existing human serum samples to validate model outputs. Given frequent SARSr-CoV spillover events into local human populations, this can be done to a degree not possible in systems where spillover events are rare.

Intervention Development: (2 parallel approaches)

- (1) Broadscale Immune Boosting Strategy: Inoculate bats with immune modulators to upregulate innate immune response and downregulate viral replication, transiently reducing risk of viral shedding and spillover.
- (2) Targeted Immune Priming Strategy: Inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance innate immune response against specific, high-risk viruses.
- Viral Dynamics: Develop stochastic simulation models to estimate the frequency, efficacy, and population coverage required for intervention approaches to effectively suppress the viral population.
- Field Deployment and Testing: Ulilze team's expertise in wildlife vaccine delivery to assess and deploy effective molecule delivery methods, including: automated aerosolization technology that inoculates bats as they leave cave roost; remote controlled drone technology; transdermal nanoparticle application; and application of edible, adhesive gels that bats ingest when grooming fur of self and others.

IMPACT

- Recent and ongoing security concerns within South and SE Asia make the region a likely deployment site for US warfighters. Troops deployed to the region face increased disease risk from SARS and related bat viruses, as bats shed these pathogens through urine and feces while foraging over large areas at night.
- Our work in Yunnan Province, China has shown that (1) SARSr-CoVs are capable of producing SARS-like illness in humanized mice that are not affected by monoclonal or vaccine treatment, and (2) that spillover into local human populations is frequent. With no available vaccine or alternative method to counter these SARS-related viruses, US defense forces and national security are placed at risk.
- Our goal is to "DEFUSE" the potential for emergence of novel bat-origin high zoonotic risk SARSr-CoVs in Southeast Asia. In doing so, we will not only safeguard the US warfighter, but also reduce SARSr-CoV exposure for local communities and their livestock, improving food security Global Health Security.
- If successful, our strategy can be adapted to hosts of other bat-origin CoVs (MERS-CoV in the Middle East and other SARS-related pre-pandemic zoonotic strains in Africa, e.g. Nigeria), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, Ebola viruses).

CONTEXT

- No technology currently exists to reduce the risk of exposure to novel bat Coronaviruses.
- Our team has conducted pioneering research on modeling disease emergence, understanding Coronavirus virology, bat immunity, and wildlife vaccine delivery. Our previous work provides proof-of-concept for: (1) predictive 'hotspot' modeling; (2) upregulating bat immune response through the STING IFN pathway, (2) developing recombinant chimeric spike-proteins from SARS and SARSr-CoVs and (3) delivering immunological countermeasures to wildlife (including multiple bat species).
- The DEFUSE approach is broadly effective, scalable, economical and achievable in the allotted time frame. It also poses little environmental risk, and presents no threat to local livestock or human populations.
- While CRISPR-Cas9 gene drives are being considered for many disease research applications, the technique is unlikely to be effective in suppressing viral transmission in bat hosts. Bats are relatively long-lived, highly mobile, and have long inter-generational periods (2-5 years) with low progeny (1-2 pups). Furthermore, gene drive technology could have far-reaching, negative ecological consequences and its effectiveness cannot be evaluated within the defined Period of Performance.

	Phase I	Phase II	Total
Proposed	$-	$-	$-

☒Human Use/ ☒ Animal Use

RE: Final draft DARPA abstract

Wang Linfa <linfa.wang@duke-nus.edu.sg>

Tue 2/13/2018 12:09 AM

To: Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

Hi Peer,

It actually reads well now and I hope the selection committee will “buy in” our brave ideas!

Nothing to add a or change, other than an English question: we used “dampened immunity of bats” in most places, but you use the expression of “damping innate immunity pathways” on page 1. Is there a difference between “damping” and “dampening”?

Thanks

LF

Linfa (Lin-Fa) Wang, PhD FTSE
Professor & Director
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8 College Road, Singapore 169875
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From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Tuesday, 13 February 2018 1:23 PM
To: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
Subject: Final draft DARPA abstract
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RE: Final draft DARPA 500 wd summary

Wang Linfa <linfa.wang@duke-nus.edu.sg>

Tue 2/13/2018 12:15 AM

To: Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

Dear Peter,

Another English questions (sorry, I seem to have all these English questions today!): you have used “inoculate” to cover all of the proposed approaches in this proposal. If we decide to “spray” chemicals for bats to breath in, can we still consider this as “inoculate”?! Or shall we be more general and used “apply” instead, by stating that “we will apply immune modulators and vaccine to bats”?!

Thanks

Linfa (Lin-Fa) Wang, PhD FTSE
Professor & Director
Programme in Emerging Infectious Diseases
Duke-NUS Medical School
8 College Road, Singapore 169875
Tel: +65 65168397

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Tuesday, 13 February 2018 2:04 PM
To: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
Subject: RE: Final draft DARPA 500 wd summary
Importance: High

It’s 497 words now.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

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www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Peter Daszak
Sent: Tuesday, February 13, 2018 12:23 AM
To: Luke Hamel (hamel@ecohealthalliance.org); Jonathon Musser
Cc: William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); wang linfa; 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura (chmura@ecohealthalliance.org); 'Anna Willoughby (willoughby@ecohealthalliance.org)'; Rocke, Tonie
Subject: Final draft DARPA abstract
Importance: High

Luke, attached is the DARPA abstract.

First thing tomorrow, can you go through the references, and create links to the papers on NCBI. Just turn each reference citation: "(*1*)" for example, into a live link to the paper on NCBI. We only have 2 spare lines, so no room to turn each of these into a PMC numbered ref, as Ralph did for his, but please make sure the citation in parentheses is blue, so it's clear it's a live link on the final pdf.

Ralph, Lina, Peng, Zhengli, Tonie – please give this a quick read to make sure I've not said anything completely wrong. I've had to reduce the text a lot to hit the page limit, but I still think it's a great proposal.

I'll finish off the exec summary slide and <500 wd abstract now.

Cheers,

Peter

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Re: Final draft abstract

peng.zhou <peng.zhou@wh.iov.cn>

Tue 2/13/2018 1:26 AM

To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; wang linfa <linfa.wang@duke-nus.edu.sg>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

Hi, Peter ,

It was really great proposal ! Just a couple of minor issues :

1, at 3, you used "spike protein", for the immune part. But you used "CoV fragments" for immunization in the actual ta2 part. Please be consistent. Also be consistent if we use CoV fragments to immunize bats or just "inoculate " (bat or human).
2, last paragraph : Duke-Nus but not Duke Nus. Prof. Zhengli Shi, rather than Dr. ?

All good for other parts!
Best wishes ,
Peng

周鹏

邮箱：peng.zhou@wh.iov.cn

在2018年02月13日 13:23，Peter Daszak 写道：

Luke, attached is the DARPA abstract.

First thing tomorrow, can you go through the references, and create links to the papers on NCBI. Just turn each reference citation: "(*1*)" for example, into a live link to the paper on NCBI. We only have 2 spare lines, so no room to turn each of these into a PMC numbered ref, as Ralph did for his, but please make sure the citation in parentheses is blue, so it's clear it's a live link on the final pdf.

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From: Rocke, Tonie <trocke@usgs.gov>
Sent: Monday, February 12, 2018 11:48 PM
To: Peter Daszak
Cc: Luke Hamel; Jonathon Musser; William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); wang linfa; 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby
Subject: Re: Final draft DARPA abstract
Attachments: DARPA PREEMPT DEFUSE abstract 3_TRedits.docx

Just minor edits. Looks good. -Tonie

On Mon, Feb 12, 2018 at 11:23 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

> Luke, attached is the DARPA abstract.
>
> First thing tomorrow, can you go through the references, and create links to the papers on NCBI. Just turn each reference citation: "(*1*)" for example, into a live link to the paper on NCBI. We only have 2 spare lines, so no room to turn each of these into a PMC numbered ref, as Ralph did for his, but please make sure the citation in parentheses is blue, so it's clear it's a live link on the final pdf.
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--
Tonie E. Rocke
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Madison, WI 53711
608-270-2451
trocke@usgs.gov

DARPA – PREEMPT – HR001118S0017- PROJECT DEFUSE

C. Goals and Impact:

1. What is the proposed work attempting to accomplish or do?

We will **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses**. We envisage a scenario whereby the US warfighter is deployed to a security hotspot in SE Asia. As planners choose sites for the mission, they will use an app we will design based on machine-learning models of the ecological and evolutionary potential of bat viruses to spillover. This will allow rapid assessment of the background risk of a site harboring dangerous zoonotic viruses. If there is no alternative site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release **broadscale immune boosting molecules** and chimeric polyvalent spike protein **targeted immune priming inocula** to upregulate the naturally damped innate immune response of bats, and lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

There is no available current technology to reduce the risk of exposure to novel coronaviruses from bats. Models of bat host capacity to harbor viruses, of ecological and environmental drivers of their emergence, and of the evolutionary potential of different strains to spillover are rudimentary. No vaccines or therapeutics exist for SARSr-CoVs, and exposure mitigation strategies are non-existent. SARSr-CoVs are endemic in Asian, African (*1*), and European bats (*2*) that roost in caves but forage widely at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARSr-CoVs into people in China and have isolated strains capable of producing SARS-like illness in humanized mice that don't respond to antibody treatment or vaccination. These viruses are a clear-and-present danger to our military and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

Our group leads the world in predictive models of viral emergence. We will build on our hotspots, machine-learning, ecological niche and genotype-phenotype modeling by incorporating unique datasets to validate and refine hotspot risk maps of viral emergene in SE Asia and beyond. Our group has shown that bats coexist with lethal viruses by damping innate immunity pathways, likely as an evolutionary adaptation to flight. We will use this insight to design strategies, like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists, to upregulate bat immunity in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover **(broadscale immune boosting strategy)**. We will complement this by inoculating bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against specific, high-risk viruses **(targeted immune priming strategy)**, especially when their immune response is boosted as above. We will design novel methods to deliver

these inocula remotely to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?
Modeling: Previous models have suffered from a lack of data to validate them. We have access to unique datasets that will allow us to validate our approach, including biodiversity surveys of bat caves across S. China, 10+ years of bat viral testing data in China, and 10 other countries (from NIAID and USAID EPT PREDICT work). Uniquely, we will validate our models of viral evolution/spillover risk using serology (based on LIPS assays) in local populations that have high (~3%) seroprevelance to bat SARSr-CoVs.
Identifying Immune boosting and priming inocula: Some of our approaches are novel and challenging (e.g. using CRISPRi to find the negative regulator for bat interferon production), and others are unproven in bats (e.g. Poly IC). We will begin all immune boosting and priming experiments at the beginning of the project, running them simultaneously and competitively, so that we field trial only the most efficient, cost-effective and scalable approaches.

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. Potential deployment to regions where SARSr-CoVs exist is high – countries include security hotspots in Asia (e.g. Myanmar, Bangladesh, Pakistan, Korea, Vietnam), Africa and Eastern Europe. The ability to decontaminate and defuse these viruses may prevent potentially devastating illness. These technologies could be adapted to hosts of other bat-origin CoVs (e.g. MERS-CoV, SADS-CoV) and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV), with benefits to livestock production, food security and global public health.

6. How much will it cost and how long will it take?
Aleksei/Luke to fill out
SPACE
SPACE
SPACE

D. Technical Plan:
Overview
The SARSr-CoV-bat system, and immune modulation focus: Our group's 15 yrs work on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014 & WIV-1). We have shown they bind and replicate efficiently in primary human lung airway cells and that chimeras with SARSr-CoV spike proteins in a SARS-CoV backbone cause SARS-like illness in humanized mice, with clinical signs that are not reduced by SARS monoclonal therapy or vaccination. We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV (*7-9*). We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover,

and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**. Our work on bat immunology suggests that bats' unique flying ability has led to downregulated innate immune genes, and their ability to coexist with viruses such as SARSr-CoVs, henipa- and filoviruses that are lethal in many other mammals (*3*). We have identified bat-specific constitutively expressed bat interferon, a dampened STING-interferon production pathway (*4, 5*), and have identified a series of other innate immunity factors that are dampened in bats (*6*).

Our bat-CoV system has significant advantages for experimentation and intervention. Firstly, these viruses are fecal-orally transmitted within bat populations, so sampling can be achieved from fresh fecal pellet collection. They are BSL-3, not -4, agents, so that experimental manipulation and infection is simpler. They have frequent spillover events, making it possible to validate predictive models of spillover by sampling people. They are diverse, with frequent recombination and different strains exhibiting differential host cell binding and spillover potential. Finally, we have identified SARSr-CoV strains in a single cave in Yunnan that harbor all of the epidemic SARS-CoV genes. This specific bat population harbors an ideal evolutionary soup that could produce new human strains by high frequency RNA recombination, and thus, it presents a perfect target for next generation, technology-forward intervention strategies.

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team, led by Drs Daszak, Ross, Olival, EHA, will build ecological niche models of environmental and ecological correlates and traits of cave bat communities to predict species composition of bat caves across Southern China, South and SE Asia. We will then use a series of datasets we have built to produce host-virus risk models for the region. These include our unique database of bat host-viral relationships (*7*); biological inventory data on all bat caves in Southern China; and modeled species distribution data for all bats. We will parameterize the model with data from three cave sites in Yunnan, China (one with high-risk SARSr-CoVs, two other control/comparison sites), including: radio- and GPS-telemetry to identify home range and additional roost sites for each bat species; inventory of bat population density, distribution and segregation and their daily, weekly and seasonal changes; viral prevalence and individual viral load; shedding of low- and high-risk SARSr-CoV strains among bat species, age classes, genders; and telemetry and mark-recapture data to assess metapopulation structure and inter-cave connectivity. We will test and validate model predictions of a cave's viral spillover potential with data from prior PREDICT sampling in 7 other Asian countries. At the end of Yr 1, we will produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens in a region. **The 'high-risk bats near me' app** will be updated real-time with surveillance data (e.g. field-deployable iphone and android compatible echolocation data) from our project and others, to ground-truth and fine-tune its predictive capacity.

The Wuhan Institute of Virology team will test bat fecal, oral, blood and urogenital samples for SARSr-CoVs. We will correlate viral load data from these samples

with fresh fecal pellets from individuals and from tarps laid on cave floors. We will rapidly move to fecal pellet assays to reduce roost disturbance. SARSr-CoV spike proteins will be sequenced, analyzed phylogenetically for recombination events, and high-risk viruses (spike proteins close to SARS-CoV) characterized and isolated. The UNC team will reverse-engineer spike proteins to conduct binding assays to human ACE2 (the SARS-CoV receptor). They will culture SARS-like bat coronaviruses to distinguish high risk strains that can replicate in primary human cells and low risk strains that require exogenous enhancers. Viral spike glycoproteins that bind receptors will be inserted into SARS-CoV backbones, inoculated into human cells and humanized mice to assess capacity to cause SARS-like disease, and to be blocked by monoclonal therapies, the nucleoside analogue inhibitor GS-5734 (*8*) or vaccines against SARS-CoV (*8-13*).

The EHA modeling team will use these data to **build models of risk of viral evolution and spillover**. These genotype-to-phenotype machine-learning models will predict viral ability to infect host cells based on genetic traits and results of receptor binding and mouse infection assays. Using data on diversity of spike proteins, recombinant CoVs, and flow of genes within each bat cave via bat movement and migration, we will estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Finally, virus-host relationship and bat home range data will be used to estimate spillover potential - extending models well beyond our field sites. We will then **validate model predictions of viral spillover risk** by **1)** conducting spike protein-based binding and cell culture experiments, and **2)** identifying spillover strains in people near our bat cave sites. Our preliminary work on this shows ~3% seroprevalence to SARSr-CoVs, using a specific ELISA (*14*). We will design LIPS assays to the specific high- and low- zoonotic-risk SARSr-CoVs identified in this project as we have done previously (*15*). We will use banked and newly collected human sera from these populations to test for presence of antibodies to the high- and low-risk SARSr-CoVs identified by our modeling. We will then **model optimal strategies to maximize inoculation efficacy** for TA2, using machine-learning stochastic simulation modeling informed by field and experimental data to characterize viral circulation dynamics in bats. We will estimate frequency and population coverage required for our intervention approaches to suppress viral spillover. We will determine the seasons, locations within a cave, and delivery methods (spray, swab, or automated cave mouth or drone) that will be most effective. Finally we will determine the time period treatment will be effective for, until re-colonization or evolution leads to return of a high-risk SARSr-CoV.

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

We will evaluate two approaches to defuse SARS-related CoV spillover potential**: 1) Broadscale Immune Boosting:** using the unique immune damping in bats that our group has discovered, we will inoculate live bats with immune modulators like bat interferon designed to up-regulate their naïve immunity and then assess their ability to suppress viral replication and shedding; **2) Targeted Immune Priming:** building on preliminary

development of polyvalent chimeric recombinant SARSr-CoV spike proteins, we will conduct inoculation trials with live bats to assess suppression of replication and shedding of a broad range of dangerous SARS-related CoVs.

Both lines of work will begin in Yr 1 and run parallel. Prof. Linfa Wang (Duke-NUS) will lead the immune boosting work, building on his pioneering work on bat immunity (*3*) which shows that the long-term coexistence of bats and their viruses has led to equilibrium between viral replication and host immunity. This is likely due to down-regulation of their innate immune system as a fitness cost of flight (*3*). The weakened functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may allow bats to maintain an effective, but not over-response to viruses (*4*). A similar finding was observed for bat IFNA, which is less abundant but constitutively expressed without stimulation (*5*). Given high native SARSr-CoV load in bats, we aim to boost bat innate immunity through the IFN pathway, break the host-virus equilibrium to suppress bat SARSr-CoV replication and shedding.

We will trial the following, concurrently and competitively, for efficiency, cost and scalability: **i)** Universal bat interferon. Aerosol spraying or intranasal inoculation of IFN or other small molecules reduces viral loads in humans, ferrets and mouse models (*16, 17*). Interferon has been used clinically when antiviral drugs are unavailable, e.g. against filoviruses (*18*). Replication of SARSr-CoV is sensitive to interferon treatments, as shown in our previous work (*16*); **ii)** Boosting bat IFN by blocking bat-specific IFN negative regulators. Uniquely, bat IFNA is naturally constitutively expressed but cannot be induced to a high level (*5*), indicating a negative regulatory factor in the bat interferon production pathway. We will use CRISPRi to identify the negative regulator and then screen for compounds targeting this gene; **iii)** Activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7-dependent pathways. Our work showing that mutant bat STING restores antiviral functionality suggests these pathways are important in bat-viral coexistence (*4*). By identifying small molecules to directly activate downstream of STING, we will activate bat interferon and promote viral clearance. A similar strategy will be applied to ssRNA-TLR7-dependent pathways; **iv)** Activating functional bat IFN production pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been demonstrated in a mouse model for SARS-CoV, IAV and HBV (*17, 19*); **v)** Inoculating crude coronavirus fragments to upregulate innate immune responses to specific CoVs – a partial step towards the targeted immune priming work below.

Prof. Ralph Baric (UNC) will lead the immune priming work. He will develop recombinant chimeric spike-proteins (*20*) from our known SARSr-CoVs, and those we characterize during project DEFUSE. The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain (*21*). In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles (*11, 22-25*). We will test these in controlled lab conditions, taking the best candidate forward for testing in the field. We have built recombinant spike glycoproteins harboring structurally

defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broadscale immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, potentially reducing disease risk (and transmission risk to people) in these animals for longer periods (*26, 27*).

The immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established the only experimental breeding colony of cave bats (*Eonycteris spelaea*) in SE Asia. This genus is evolutionarily closely related to *Rhinolophus* spp. (the hosts of SARSr-CoVs), so we have confidence that results will be transferable. Our initial proof-of-concept tests will be in this experimental colony, extended to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with *Rhinolophus* sp. bats in the BSL-4 facility at CSIRO, AAHL (L.Wang, unpublished results).

Finally, work on a delivery method for our immune boosting and priming molecules will be overseen by Dr. Tonie Rocke at the USGS, National Wildlife Health Center who has previously developed animal vaccines through to licensure (*28*). Using locally acquired insectivorous bats (*29, 30*) we will assess delivery vehicles and methods including: 1) transdermally applied nanoparticles; 2) series of sticky edible gels that bats will groom from themselves and each other; 3) aerosolization via sprayers that could be used in cave settings; 4) automated sprays triggered by timers and movement detectors at critical cave entry points; 5) sprays delivered by remote controlled drone. We have already used simple gels to vaccinate bats against rabies in the lab (*29*), and hand delivered these containing biomarkers to vampire bats in Peru and Mexico to show they are readily consumed and transferred among bats. In our bat colony, we will trial delivery vehicles using the biomarker rhodamine B (which marks hair and whiskers upon consumption) to assess uptake. The most optimal approaches will then be tested on wild bats in our three cave sites in Yunnan Province with the most successful immunomodulators from TA2. Fieldwork will be conducted under the auspices of Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance). A small number of bats will be captured and assayed for viral load and immune function after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has had unique access to these sites for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for experimental inoculations from the Provincial Forestry Department. We expect to be successful, as we have worked with the Forestry Department collaboratively for 10 years, with support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife. EHA has a proven track record of rapidly obtaining IACUC and DoD ACURO approval for bat research.

E. Capabilities:

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research organization focused on emerging zoonotic diseases. The PI, Dr. Peter Daszak, has 25+ years' experience managing lab, field and modeling research projects on

emerging zoonoses. Dr. Daszak will commit 3 months annually to oversee and coordinate all project activities, and lead modeling and analytic work for TA1. Dr. Billy Karesh has 40+ years' experience leading zoonotic and wildlife disease projects, and will commit 1 month annually to manage partnership activities and outreach. Dr. Jon Epstein, with 15 years' experience working emerging bat zoonoses will coordinate animal trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project. Support staff include field surveillance teams, modeling analysts, and consultants based in Yunnan Province, China, to oversee field trials. The EHA team has worked extensively with all other collaborators: Prof. Wang (15+ years); Dr. Shi (15+ years); Prof. Baric (5+ years) and Dr. Rocke (15+ years).

Subcontracts: #1 to Prof. Ralph Baric, UNC, to oversee reverse engineering of SARSr-CoVs, BSL-3 humanized mouse experimental infections, design and testing of immune priming inocula based on recombinant spike proteins. Assisted by senior personnel Dr. Tim Sheahan, Dr. Amy Sims, and support staff; **#2** to Prof. Linfa Wang, Duke NUS, to oversee the immune boosting approach, captive bat experiments, and analyze immunological and virological responses to immune boosting inocula; **#3** to Dr. Zhengli Shi, Wuhan Institute of Virology, to conduct PCR testing, viral discovery and isolation from bat samples collected in China, spike protein binding assays, and some humanized mouse work, as well as experimental inoculation of *Rhinolophus* bats. Her team will include Dr. Peng Zhou and support staff; **#4** to Dr. Tonie Rocke, USGS National Wildlife Health Center, to refine delivery mechanisms for both immune boosting and immune priming treatments. With a research technician, Dr. Rocke will use a captive colony of bats at NWHC for initial trials, and oversee cave experiments in China.

F. *Links to published papers, resume of two key performers*

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based research organization focused on emerging zoonotic diseases. His >300 scientific papers include the first global map of EID hotspots (*31, 32*), estimates of unknown viral diversity (*33*), predictive models of virus-host relationships (*7*), and evidence of the bat origin of SARS-CoV (*34, 35*) and other emerging viruses (*36-39*). He is Chair of the NASEM Forum on Microbial Threats, and is a member of the Executive Committee and the EHA institutional lead for the $130 million USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Dept. of Epidemiology and Dept. of Microbiology & Immunology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, cross species transmission and pathogenesis. His group has developed a platform strategy to access the potential "pre-epidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (*8-13*).

Citations

1. P. L. Quan *et al.*, Identification of a Severe Acute Respiratory Syndrome Coronavirus-Like Virus in a Leaf-Nosed Bat in Nigeria. *Mbio* **1**, (2010).
2. J. F. Drexler *et al.*, Genomic Characterization of Severe Acute Respiratory Syndrome-Related Coronavirus in European Bats and Classification of Coronaviruses Based on Partial RNA-Dependent RNA Polymerase Gene Sequences. *Journal of Virology* **84**, 11336-11349 (2010).
3. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
4. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
5. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
6. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
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Re: Final draft DARPA abstract

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Tue 2/13/2018 5:22 AM

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Cc: Jonathon Musser <musser@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; wang linfa <linfa.wang@duke-nus.edu.sg>; Rocke, Tonie E <trocke@usgs.gov>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>

Added just a couple of small edits on modeling phrasing, done on top of Tonie's edits.

On Tue, Feb 13, 2018 at 2:47 AM Rocke, Tonie <trocke@usgs.gov> wrote:

> Just minor edits. Looks good. -Tonie
>
> On Mon, Feb 12, 2018 at 11:23 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:
>
> > Luke, attached is the DARPA abstract.
> >
> > First thing tomorrow, can you go through the references, and create links to the papers on NCBI. Just turn each reference citation: "(*1*)" for example, into a live link to the paper on NCBI. We only have 2 spare lines, so no room to turn each of these into a PMC numbered ref, as Ralph did for his, but please make sure the citation in parentheses is blue, so it's clear it's a live link on the final pdf.
> >
> > Ralph, Lina, Peng, Zhengli, Tonie – please give this a quick read to make sure I've not said anything completely wrong. I've had to reduce the text a lot to hit the page limit, but I still think it's a great proposal.
> >
> > I'll finish off the exec summary slide and <500 wd abstract now.
> >
> > Cheers,
> >
> > Peter

Peter Daszak

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

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DARPA – PREEMPT – HR001118S0017- PROJECT DEFUSE

C. Goals and Impact:

1. What is the proposed work attempting to accomplish or do?

We will **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses**. We envisage a scenario whereby the US warfighter is deployed to a security hotspot in SE Asia. As planners choose sites for the mission, they will use an app we will design based on machine-learning models of the ecological and evolutionary potential of bat viruses to spillover. This will allow rapid assessment of the background risk of a site harboring dangerous zoonotic viruses. If there is no alternative site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release **broadscale immune boosting molecules** and chimeric polyvalent spike protein **targeted immune priming inocula** to upregulate the naturally damped innate immune response of bats, and lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

There is no available current technology to reduce the risk of exposure to novel coronaviruses from bats. Models of bat host capacity to harbor viruses, of ecological and environmental drivers of their emergence, and of the evolutionary potential of different strains to spillover are rudimentary. No vaccines or therapeutics exist for SARSr-CoVs, and exposure mitigation strategies are non-existent. SARSr-CoVs are endemic in Asian, African (*1*), and European bats (*2*) that roost in caves but forage widely at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARSr-CoVs into people in China and have isolated strains capable of producing SARS-like illness in humanized mice that don't respond to antibody treatment or vaccination. These viruses are a clear-and-present danger to our military and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

Our group leads the world in predictive models of viral emergence. We will build on our machine-learning models of spillover hotspots, host-pathogen ecological niches and genotype-phenotype mapping by incorporating unique datasets to validate and refine hotspot risk maps of viral emergence in SE Asia and beyond. Our group has shown that bats coexist with lethal viruses by damping innate immunity pathways, likely as an evolutionary adaptation to flight. We will use this insight to design strategies, like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists, to upregulate bat immunity in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover **(broadscale immune boosting strategy)**. We will complement this by inoculating bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against specific, high-risk viruses **(targeted immune priming strategy)**, especially when their immune response is boosted as above.

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We will design novel methods to deliver these inocula remotely to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?
Modeling: Previous models have suffered from a lack of data to validate them. We have access to unique datasets that will allow us to validate our approach, including biodiversity surveys of bat caves across S. China, 10+ years of bat viral testing data in China, and 10 other countries (from NIAID and USAID EPT PREDICT work). Uniquely, we will validate our models of viral evolution/spillover risk using serology (based on LIPS assays) in local populations that have high (~3%) seroprevelance to bat SARSr-CoVs.
Identifying Immune boosting and priming inocula: Some of our approaches are novel and challenging (e.g. using CRISPRi to find the negative regulator for bat interferon production), and others are unproven in bats (e.g. Poly IC). We will begin all immune boosting and priming experiments at the beginning of the project, running them simultaneously and competitively, so that we field trial only the most efficient, cost-effective and scalable approaches.

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5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. Potential deployment to regions where SARSr-CoVs exist is high – countries include security hotspots in Asia (e.g. Myanmar, Bangladesh, Pakistan, Korea, Vietnam), Africa and Eastern Europe. The ability to decontaminate and defuse these viruses may prevent potentially devastating illness. These technologies could be adapted to hosts of other bat-origin CoVs (e.g. MERS-CoV, SADS-CoV) and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV), with benefits to livestock production, food security and global public health.

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6. How much will it cost and how long will it take?
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D. Technical Plan:
Overview
The SARSr-CoV-bat system, and immune modulation focus: Our group's 15 yrs work on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014 & WIV-1). We have shown they bind and replicate efficiently in primary human lung airway cells and that chimeras with SARSr-CoV spike proteins in a SARS-CoV backbone cause SARS-like illness in humanized mice, with clinical signs that are not reduced by SARS monoclonal therapy or vaccination. We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV (*7-9*). We have now shown that people living up to 6 kilometers from this cave have

SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**. Our work on bat immunology suggests that bats' unique flying ability has led to downregulated innate immune genes, and their ability to coexist with viruses such as SARSr-CoVs, henipa- and filoviruses that are lethal in many other mammals (*3*). We have identified bat-specific constitutively expressed bat interferon, a dampened STING-interferon production pathway (*4, 5*), and have identified a series of other innate immunity factors that are dampened in bats (*6*).

Our bat-CoV system has significant advantages for experimentation and intervention. Firstly, these viruses are fecal-orally transmitted within bat populations, so sampling can be achieved from fresh fecal pellet collection. They are BSL-3, not -4, agents, so that experimental manipulation and infection is simpler. They have frequent spillover events, making it possible to validate predictive models of spillover by sampling people. They are diverse, with frequent recombination and different strains exhibiting differential host cell binding and spillover potential. Finally, we have identified SARSr-CoV strains in a single cave in Yunnan that harbor all of the epidemic SARS-CoV genes. This specific bat population harbors an ideal evolutionary soup that could produce new human strains by high frequency RNA recombination, and thus, it presents a perfect target for next generation, technology-forward intervention strategies.

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team, led by Drs Daszak, Ross, Olival, EHA, will build ecological niche models of environmental and ecological correlates and traits of cave bat communities to predict species composition of bat caves across Southern China, South and SE Asia. We will then use a series of datasets we have built to produce host-virus risk models for the region. These include our unique database of bat host-viral relationships (*7*); biological inventory data on all bat caves in Southern China; and modeled species distribution data for all bats. We will parameterize the model with data from three cave sites in Yunnan, China (one with high-risk SARSr-CoVs, two other control/comparison sites), including: radio- and GPS-telemetry to identify home range and additional roost sites for each bat species; inventory of bat population density, distribution and segregation and their daily, weekly and seasonal changes; viral prevalence and individual viral load; shedding of low- and high-risk SARSr-CoV strains among bat species, age classes, genders; and telemetry and mark-recapture data to assess metapopulation structure and inter-cave connectivity. We will test and validate model predictions of a cave's viral spillover potential with data from prior PREDICT sampling in 7 other Asian countries. At the end of Yr 1, we will produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens in a region. **The 'high-risk bats near me' app** will be updated real-time with surveillance data (e.g. field-deployable iphone and android compatible echolocation data) from our project and others, to ground-truth and fine-tune its predictive capacity.

The Wuhan Institute of Virology team will test bat fecal, oral, blood and

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urogenital samples for SARSr-CoVs. We will correlate viral load data from these samples with fresh fecal pellets from individuals and from tarps laid on cave floors. We will rapidly move to fecal pellet assays to reduce roost disturbance. SARSr-CoV spike proteins will be sequenced, analyzed phylogenetically for recombination events, and high-risk viruses (spike proteins close to SARS-CoV) characterized and isolated. The UNC team will reverse engineer spike proteins to conduct binding assays to human ACE2 (the SARS-CoV receptor). They will culture SARS-like bat coronaviruses to distinguish high risk strains that can replicate in primary human cells and low risk strains that require exogenous enhancers. Viral spike glycoproteins that bind receptors will be inserted into SARS-CoV backbones, inoculated into human cells and humanized mice to assess capacity to cause SARS-like disease, and to be blocked by monoclonal therapies, the nucleoside analogue inhibitor GS-5734 (*8*) or vaccines against SARS-CoV (*8-13*).

The EHA modeling team will use these data to **build models of risk of viral evolution and spillover**. These genotype-to-phenotype machine-learning models will predict viral ability to infect host cells based on genetic traits and results of receptor binding and mouse infection assays. Using data on diversity of spike proteins, recombinant CoVs, and flow of genes within each bat cave via bat movement and migration, we will estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Finally, virus-host relationship and bat home range data will be used to estimate spillover potential - extending models well beyond our field sites. We will then **validate model predictions of viral spillover risk** by **1)** conducting spike protein-based binding and cell culture experiments, and **2)** identifying spillover strains in people near our bat cave sites. Our preliminary work on this shows ~3% seroprevalence to SARSr-CoVs, using a specific ELISA (*14*). We will design LIPS assays to the specific high- and low- zoonotic-risk SARSr-CoVs identified in this project as we have done previously (*15*). We will use banked and newly collected human sera from these populations to test for presence of antibodies to the high- and low-risk SARSr-CoVs identified by our modeling. We will then **model optimal strategies to maximize inoculation efficacy** for TA2, using stochastic simulation modeling informed by field and experimental data to characterize viral circulation dynamics in bats. We will estimate frequency and population coverage required for our intervention approaches to suppress viral spillover. We will determine the seasons, locations within a cave, and delivery methods (spray, swab, or automated cave mouth or drone) that will be most effective. Finally we will determine the time period treatment will be effective for, until re-colonization or evolution leads to return of a high-risk SARSr-CoV.

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

We will evaluate two approaches to defuse SARS-related CoV spillover potential**: 1) Broadscale Immune Boosting:** using the unique immune damping in bats that our group has discovered, we will inoculate live bats with immune modulators like bat interferon designed to up-regulate their naïve immunity and then assess their ability to suppress viral replication and shedding; **2) Targeted Immune Priming:** building on preliminary

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development of polyvalent chimeric recombinant SARSr-CoV spike proteins, we will conduct inoculation trials with live bats to assess suppression of replication and shedding of a broad range of dangerous SARS-related CoVs.

Both lines of work will begin in Yr 1 and run parallel. Prof. Linfa Wang (Duke-NUS) will lead the immune boosting work, building on his pioneering work on bat immunity (*3*) which shows that the long-term coexistence of bats and their viruses has led to equilibrium between viral replication and host immunity. This is likely due to down-regulation of their innate immune system as a fitness cost of flight (*3*). The weakened functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may allow bats to maintain an effective, but not over-response to viruses (*4*). A similar finding was observed for bat IFNA, which is less abundant but constitutively expressed without stimulation (*5*). Given high native SARSr-CoV load in bats, we aim to boost bat innate immunity through the IFN pathway, break the host-virus equilibrium to suppress bat SARSr-CoV replication and shedding.

We will trial the following, concurrently and competitively, for efficiency, cost and scalability: **i)** Universal bat interferon. Aerosol spraying or intranasal inoculation of IFN or other small molecules reduces viral loads in humans, ferrets and mouse models (*16, 17*). Interferon has been used clinically when antiviral drugs are unavailable, e.g. against filoviruses (*18*). Replication of SARSr-CoV is sensitive to interferon treatments, as shown in our previous work (*16*); **ii)** Boosting bat IFN by blocking bat-specific IFN negative regulators. Uniquely, bat IFNA is naturally constitutively expressed but cannot be induced to a high level (*5*), indicating a negative regulatory factor in the bat interferon production pathway. We will use CRISPRi to identify the negative regulator and then screen for compounds targeting this gene; **iii)** Activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7-dependent pathways. Our work showing that mutant bat STING restores antiviral functionality suggests these pathways are important in bat-viral coexistence (*4*). By identifying small molecules to directly activate downstream of STING, we will activate bat interferon and promote viral clearance. A similar strategy will be applied to ssRNA-TLR7-dependent pathways; **iv)** Activating functional bat IFN production pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been demonstrated in a mouse model for SARS-CoV, IAV and HBV (*17, 19*); **v)** Inoculating crude coronavirus fragments to upregulate innate immune responses to specific CoVs – a partial step towards the targeted immune priming work below.

Prof. Ralph Baric (UNC) will lead the immune priming work. He will develop recombinant chimeric spike-proteins (*20*) from our known SARSr-CoVs, and those we characterize during project DEFUSE. The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain (*21*). In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles (*11, 22-25*). We will test these in controlled lab conditions, taking the best candidate forward for testing in the field. We have built recombinant spike glycoproteins harboring structurally

defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broadscale immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, potentially reducing disease risk (and transmission risk to people) in these animals for longer periods (*26, 27*).

The immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established the only experimental breeding colony of cave bats (*Eonycteris spelaea*) in SE Asia. This genus is evolutionarily closely related to *Rhinolophus* spp. (the hosts of SARSr-CoVs), so we have confidence that results will be transferable. Our initial proof-of-concept tests will be in this experimental colony, extended to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with *Rhinolophus* sp. bats in the BSL-4 facility at CSIRO, AAHL (L.Wang, unpublished results).

Finally, work on a delivery method for our immune boosting and priming molecules will be overseen by Dr. Tonie Rocke at the USGS, National Wildlife Health Center who has previously developed animal vaccines through to licensure (*28*). Using locally acquired insectivorous bats (*29, 30*) we will assess delivery vehicles and methods including: 1) transdermally applied nanoparticles; 2) series of sticky edible gels that bats will groom from themselves and each other; 3) aerosolization via sprayers that could be used in cave settings; 4) automated sprays triggered by timers and movement detectors at critical cave entry points; 5) sprays delivered by remote controlled drone. We have already used simple gels to vaccinate bats against rabies in the lab (*29*), and hand delivered these containing biomarkers to vampire bats in Peru and Mexico to show they are readily consumed and transferred among bats. In our bat colony, we will trial delivery vehicles using the biomarker rhodamine B (which marks hair and whiskers upon consumption) to assess uptake. The most optimal approaches will then be tested on wild bats in our three cave sites in Yunnan Province with the most successful immunomodulators from TA2. Fieldwork will be conducted under the auspices of Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance). A small number of bats will be captured and assayed for viral load and immune function after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has had unique access to these sites for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for experimental inoculations from the Provincial Forestry Department. We expect to be successful, as we have worked with the Forestry Department collaboratively for 10 years, with support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife. EHA has a proven track record of rapidly obtaining IACUC and DoD ACURO approval for bat research.

E. Capabilities:

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research organization focused on emerging zoonotic diseases. The PI, Dr. Peter Daszak, has 25+ years' experience managing lab, field and modeling research projects on

emerging zoonoses. Dr. Daszak will commit 3 months annually to oversee and coordinate all project activities, and lead modeling and analytic work for TA1. Dr. Billy Karesh has 40+ years' experience leading zoonotic and wildlife disease projects, and will commit 1 month annually to manage partnership activities and outreach. Dr. Jon Epstein, with 15 years' experience working emerging bat zoonoses will coordinate animal trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project. Support staff include field surveillance teams, modeling analysts, and consultants based in Yunnan Province, China, to oversee field trials. The EHA team has worked extensively with all other collaborators: Prof. Wang (15+ years); Dr. Shi (15+ years); Prof. Baric (5+ years) and Dr. Rocke (15+ years).

Subcontracts: #1 to Prof. Ralph Baric, UNC, to oversee reverse engineering of SARSr-CoVs, BSL-3 humanized mouse experimental infections, design and testing of immune priming inocula based on recombinant spike proteins. Assisted by senior personnel Dr. Tim Sheahan, Dr. Amy Sims, and support staff; **#2** to Prof. Linfa Wang, Duke NUS, to oversee the immune boosting approach, captive bat experiments, and analyze immunological and virological responses to immune boosting inocula; **#3** to Dr. Zhengli Shi, Wuhan Institute of Virology, to conduct PCR testing, viral discovery and isolation from bat samples collected in China, spike protein binding assays, and some humanized mouse work, as well as experimental inoculation of *Rhinolophus* bats. Her team will include Dr. Peng Zhou and support staff; **#4** to Dr. Tonie Rocke, USGS National Wildlife Health Center, to refine delivery mechanisms for both immune boosting and immune priming treatments. With a research technician, Dr. Rocke will use a captive colony of bats at NWHC for initial trials, and oversee cave experiments in China.

F. *Links to published papers, resume of two key performers*

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based research organization focused on emerging zoonotic diseases. His >300 scientific papers include the first global map of EID hotspots (*31, 32*), estimates of unknown viral diversity (*33*), predictive models of virus-host relationships (*7*), and evidence of the bat origin of SARS-CoV (*34, 35*) and other emerging viruses (*36-39*). He is Chair of the NASEM Forum on Microbial Threats, and is a member of the Executive Committee and the EHA institutional lead for the $130 million USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Dept. of Epidemiology and Dept. of Microbiology & Immunology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, cross species transmission and pathogenesis. His group has developed a platform strategy to access the potential "pre-epidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (*8-13*).

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RE: Final draft DARPA abstract

Peter Daszak <daszak@ecohealthalliance.org>

Tue 2/13/2018 7:21 AM

To: Wang Linfa <linfa.wang@duke-nus.edu.sg>; Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

Good point Linfa – changed to 'treatment' or 'application' throughout.

Cheers,

Peter

Peter Daszak
President

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www.ecohealthalliance.org

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From: Wang Linfa [mailto:linfa.wang@duke-nus.edu.sg]
Sent: Tuesday, February 13, 2018 1:10 AM
To: Peter Daszak; Luke Hamel; Jonathon Musser
Cc: William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby; Rocke, Tonie
Subject: RE: Final draft DARPA abstract

Hi Peer,

It actually reads well now and I hope the selection committee will "buy in" our brave ideas!

Nothing to add a or change, other than an English question: we used "dampened immunity of bats" in most places, but you use the expression of "damping innate immunity pathways" on page 1. Is there a difference between "damping" and "dampening"?

Thanks

LF

Linfa (Lin-Fa) Wang, PhD FTSE
Professor & Director
Programme in Emerging Infectious Diseases
Duke-NUS Medical School
8 College Road, Singapore 169875
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From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Tuesday, 13 February 2018 1:23 PM
To: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
Subject: Final draft DARPA abstract
Importance: High

Luke, attached is the DARPA abstract.

First thing tomorrow, can you go through the references, and create links to the papers on NCBI. Just turn each reference citation: "(*1*)" for example, into a live link to the paper on NCBI. We only have 2 spare lines, so no room to turn each of these into a PMC numbered ref, as Ralph did for his, but please make sure the citation in parentheses is blue, so it's clear it's a live link on the final pdf.

Ralph, Lina, Peng, Zhengli, Tonie – please give this a quick read to make sure I've not said anything completely wrong. I've had to reduce the text a lot to hit the page limit, but I still think it's a great proposal.

I'll finish off the exec summary slide and <500 wd abstract now.

Cheers,

Peter

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conservation.

RE: Final draft abstract

Peter Daszak <daszak@ecohealthalliance.org>

Tue 2/13/2018 7:21 AM

To: peng.zhou <peng.zhou@wh.iov.cn>
Cc: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; wang linfa <linfa.wang@duke-nus.edu.sg>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

Thanks Peng – made the changes now.

Cheers,

Peter

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From: peng.zhou [mailto:peng.zhou@wh.iov.cn]
Sent: Tuesday, February 13, 2018 2:26 AM
To: Peter Daszak
Cc: Luke Hamel; Jonathon Musser; William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); wang linfa; Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby; Rocke, Tonie
Subject: Re: Final draft abstract

Hi, Peter ,

It was really great proposal ! Just a couple of minor issues :

1, at 3, you used "spike protein", for the immune part. But you used "CoV fragments" for immunization in the actual ta2 part. Please be consistent. Also be consistent if we use CoV fragments to immunize bats or just "inoculate " (bat or human).
2, last paragraph : Duke-Nus but not Duke Nus. Prof. Zhengli Shi, rather than Dr. ?

All good for other parts!
Best wishes ,
Peng

周鹏

邮箱：peng.zhou@wh.iov.cn

在2018年02月13日 13:23， Peter Daszak 写道：

Luke, attached is the DARPA abstract.

First thing tomorrow, can you go through the references, and create links to the papers on NCBI. Just turn each reference citation: "(*1*)" for example, into a live link to the paper on NCBI. We only have 2 spare lines, so no room to turn each of these into a PMC numbered ref, as Ralph did for his, but please make sure the citation in parentheses is blue, so it's clear it's a live link on the final pdf.

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RE: Final draft DARPA abstract

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Tue 2/13/2018 7:21 AM

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Cc: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; wang linfa <linfa.wang@duke-nus.edu.sg>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>

Great – thanks Tonie and Noam – all changes made now..

Cheers,

Peter

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From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Tuesday, February 13, 2018 2:48 AM
To: Peter Daszak
Cc: Luke Hamel; Jonathon Musser; William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); wang linfa; 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby
Subject: Re: Final draft DARPA abstract

Just minor edits. Looks good. -Tonie

On Mon, Feb 12, 2018 at 11:23 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:
Luke, attached is the DARPA abstract.

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--
Tonie E. Rocke
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RE: Final draft DARPA abstract

Peter Daszak <daszak@ecohealthalliance.org>

Tue 2/13/2018 7:21 AM

To: Noam Ross <ross@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
Cc: Jonathon Musser <musser@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; wang linfa <linfa.wang@duke-nus.edu.sg>; Rocke, Tonie E <trocke@usgs.gov>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>

Here's the Abstract version 4 for your files.

Luke – please do the following ASAP today:

1. Fix the references – remove the endnote links, and use the format: "(*1,2*)", but making these blue, and an embedded hyperlink to the NCBI paper
2. Insert the corrected figure
3. Make sure we have the right format throughout – including grant number etc. at the top
4. Insert the timeline and budget
5. Send it in!

Cheers,

Peter

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From: Noam Ross [mailto:ross@ecohealthalliance.org]
Sent: Tuesday, February 13, 2018 6:22 AM
To: Peter Daszak; Luke Hamel
Cc: Jonathon Musser; William B. Karesh; Ralph Baric (rbaric@email.unc.edu); wang linfa; Rocke, Tonie; 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby
Subject: Re: Final draft DARPA abstract

Added just a couple of small edits on modeling phrasing, done on top of Tonie's edits.

On Tue, Feb 13, 2018 at 2:47 AM Rocke, Tonie <trocke@usgs.gov> wrote:

> Just minor edits. Looks good. -Tonie
>
> On Mon, Feb 12, 2018 at 11:23 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:
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> --
> Tonie E. Rocke
> USGS National Wildlife Health Center
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--
Dr. Noam Ross
Senior Research Scientist

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DARPA – PREEMPT – HR001118S0017- PROJECT DEFUSE

C. Goals and Impact:

1. What is the proposed work attempting to accomplish or do?

We will **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses**. We envisage a scenario whereby the US warfighter is deployed to a security hotspot in SE Asia. As planners choose sites for the mission, they will use an app we will design based on machine-learning models of the ecological and evolutionary potential of bat viruses to spillover. This will allow rapid assessment of the background risk of a site harboring dangerous zoonotic viruses. If there is no alternative site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release **broadscale immune boosting molecules** and chimeric polyvalent spike protein **targeted immune priming treatments** to upregulate the naturally damped innate immune response of bats, and lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

There is no available current technology to reduce the risk of exposure to novel coronaviruses from bats. Models of bat host capacity to harbor viruses, of ecological and environmental drivers of their emergence, and of the evolutionary potential of different strains to spillover are rudimentary. No vaccines or therapeutics exist for SARSr-CoVs, and exposure mitigation strategies are non-existent. SARSr-CoVs are endemic in Asian, African (*1*), and European bats (*2*) that roost in caves but forage widely at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARSr-CoVs into people in China and have isolated strains capable of producing SARS-like illness in humanized mice that don't respond to antibody treatment or vaccination. These viruses are a clear-and-present danger to our military and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

Our group leads the world in predictive models of viral emergence. We will build on our machine-learning models of spillover hotspots, host-pathogen ecological niches and genotype-phenotype mapping by incorporating unique datasets to validate and refine hotspot risk maps of viral emergence in SE Asia and beyond. Our group has shown that bats coexist with lethal viruses by damping innate immunity pathways, likely as an evolutionary adaptation to flight. We will use this insight to design strategies, like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists, to upregulate bat immunity in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover **(broadscale immune boosting strategy)**. We will complement this by treating bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against specific, high-risk viruses **(targeted immune priming strategy)**, especially when their immune response is boosted as above.

We will design novel methods to deliver these applications remotely to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?
Modeling: Previous models have suffered from a lack of data to validate them. We have access to unique datasets that will allow us to validate our approach, including biodiversity surveys of bat caves across S. China, 10+ years of bat viral testing data in China, and 10 other countries (from NIAID and USAID EPT PREDICT work). Uniquely, we will validate our models of viral evolution/spillover risk using serology (based on LIPS assays) in local populations that have high (~3%) seroprevelance to bat SARSr-CoVs.
Identifying Immune boosting and priming treatments: Some of our approaches are novel and challenging (e.g. using CRISPRi to find the negative regulator for bat interferon production), and others are unproven in bats (e.g. Poly IC). We will begin all immune boosting and priming experiments at the beginning of the project, running them simultaneously and competitively, so that we field trial only the most efficient, cost-effective and scalable approaches.

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. Potential deployment to regions where SARSr-CoVs exist is high – countries include security hotspots in Asia (e.g. Myanmar, Bangladesh, Pakistan, Korea, Vietnam), Africa and Eastern Europe. The ability to decontaminate and defuse these viruses may prevent potentially devastating illness. These technologies could be adapted to hosts of other bat-origin CoVs (e.g. MERS-CoV, SADS-CoV) and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV), with benefits to livestock production, food security and global public health.

6. How much will it cost and how long will it take?
Aleksei/Luke to fill out
SPACE
SPACE
SPACE

D. Technical Plan:
Overview
The SARSr-CoV-bat system, and immune modulation focus: Our group's 15 yrs work on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014 & WIV-1). We have shown they bind and replicate efficiently in primary human lung airway cells and that chimeras with SARSr-CoV spike proteins in a SARS-CoV backbone cause SARS-like illness in humanized mice, with clinical signs that are not reduced by SARS monoclonal therapy or vaccination. We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV (*7-9*). We have now shown that people living up to 6 kilometers from this cave have

SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**. Our work on bat immunology suggests that bats' unique flying ability has led to downregulated innate immune genes, and their ability to coexist with viruses such as SARSr-CoVs, henipa- and filoviruses that are lethal in many other mammals (*3*). We have identified bat-specific constitutively expressed bat interferon, a dampened STING-interferon production pathway (*4, 5*), and have identified a series of other innate immunity factors that are dampened in bats (*6*).

Our bat-CoV system has significant advantages for experimentation and intervention. Firstly, these viruses are fecal-orally transmitted within bat populations, so sampling can be achieved from fresh fecal pellet collection. They are BSL-3, not -4, agents, so that experimental manipulation and infection is simpler. They have frequent spillover events, making it possible to validate predictive models of spillover by sampling people. They are diverse, with frequent recombination and different strains exhibiting differential host cell binding and spillover potential. Finally, we have identified SARSr-CoV strains in a single cave in Yunnan that harbor all of the epidemic SARS-CoV genes. This specific bat population harbors an ideal evolutionary soup that could produce new human strains by high frequency RNA recombination, and thus, it presents a perfect target for next generation, technology-forward intervention strategies.

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team, led by Drs Daszak, Ross, Olival, EHA, will build ecological niche models of environmental and ecological correlates and traits of cave bat communities to predict species composition of bat caves across Southern China, South and SE Asia. We will then use a series of datasets we have built to produce host-virus risk models for the region. These include our unique database of bat host-viral relationships (*7*); biological inventory data on all bat caves in Southern China; and modeled species distribution data for all bats. We will parameterize the model with data from three cave sites in Yunnan, China (one with high-risk SARSr-CoVs, two other control/comparison sites), including: radio- and GPS-telemetry to identify home range and additional roost sites for each bat species; inventory of bat population density, distribution and segregation and their daily, weekly and seasonal changes; viral prevalence and individual viral load; shedding of low- and high-risk SARSr-CoV strains among bat species, age classes, genders; and telemetry and mark-recapture data to assess metapopulation structure and inter-cave connectivity. We will test and validate model predictions of a cave's viral spillover potential with data from prior PREDICT sampling in 7 other Asian countries. At the end of Yr 1, we will produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens in a region. **The 'high-risk bats near me' app** will be updated real-time with surveillance data (e.g. field-deployable iphone and android compatible echolocation data) from our project and others, to ground-truth and fine-tune its predictive capacity.

The Wuhan Institute of Virology team will test bat fecal, oral, blood and

urogenital samples for SARSr-CoVs. We will correlate viral load data from these samples with fresh fecal pellets from individuals and from tarps laid on cave floors. We will rapidly move to fecal pellet assays to reduce roost disturbance. SARSr-CoV spike proteins will be sequenced, analyzed phylogenetically for recombination events, and high-risk viruses (spike proteins close to SARS-CoV) characterized and isolated. The UNC team will reverse-engineer spike proteins to conduct binding assays to human ACE2 (the SARS-CoV receptor). They will culture SARS-like bat coronaviruses to distinguish high risk strains that can replicate in primary human cells and low risk strains that require exogenous enhancers. Viral spike glycoproteins that bind receptors will be inserted into SARS-CoV backbones, inoculated into human cells and humanized mice to assess capacity to cause SARS-like disease, and to be blocked by monoclonal therapies, the nucleoside analogue inhibitor GS-5734 (*8*) or vaccines against SARS-CoV (*8-13*).

The EHA modeling team will use these data to **build models of risk of viral evolution and spillover**. These genotype-to-phenotype machine-learning models will predict viral ability to infect host cells based on genetic traits and results of receptor binding and mouse infection assays. Using data on diversity of spike proteins, recombinant CoVs, and flow of genes within each bat cave via bat movement and migration, we will estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Finally, virus-host relationship and bat home range data will be used to estimate spillover potential - extending models well beyond our field sites. We will then **validate model predictions of viral spillover risk** by **1)** conducting spike protein-based binding and cell culture experiments, and **2)** identifying spillover strains in people near our bat cave sites. Our preliminary work on this shows ~3% seroprevalence to SARSr-CoVs, using a specific ELISA (*14*). We will design LIPS assays to the specific high- and low- zoonotic-risk SARSr-CoVs identified in this project as we have done previously (*15*). We will use banked and newly collected human sera from these populations to test for presence of antibodies to the high- and low-risk SARSr-CoVs identified by our modeling. We will then **model optimal strategies to maximize treatment efficacy** for TA2, using stochastic simulation modeling informed by field and experimental data to characterize viral circulation dynamics in bats. We will estimate frequency and population coverage required for our intervention approaches to suppress viral spillover. We will determine the seasons, locations within a cave, and delivery methods (spray, swab, or automated cave mouth or drone) that will be most effective. Finally we will determine the time period treatment will be effective for, until re-colonization or evolution leads to return of a high-risk SARSr-CoV.

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

We will evaluate two approaches to defuse SARS-related CoV spillover potential**: 1) Broadscale Immune Boosting:** using the unique immune damping in bats that our group has discovered, we will apply immune modulators like bat interferon to live bats, to up-regulate their naïve immunity and then assess their ability to suppress viral replication and shedding; **2) Targeted Immune Priming:** building on preliminary development of

polyvalent chimeric recombinant SARSr-CoV spike proteins, we will conduct application trials with live bats to assess suppression of replication and shedding of a broad range of dangerous SARS-related CoVs.

Both lines of work will begin in Yr 1 and run parallel. Prof. Linfa Wang (Duke-NUS) will lead the immune boosting work, building on his pioneering work on bat immunity (*3*) which shows that the long-term coexistence of bats and their viruses has led to equilibrium between viral replication and host immunity. This is likely due to down-regulation of their innate immune system as a fitness cost of flight (*3*). The weakened functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may allow bats to maintain an effective, but not over-response to viruses (*4*). A similar finding was observed for bat IFNA, which is less abundant but constitutively expressed without stimulation (*5*). Given high native SARSr-CoV load in bats, we aim to boost bat innate immunity through the IFN pathway, break the host-virus equilibrium to suppress bat SARSr-CoV replication and shedding.

We will trial the following, concurrently and competitively, for efficiency, cost and scalability: **i)** Universal bat interferon. Aerosol spraying or intranasal application of IFN or other small molecules reduces viral loads in humans, ferrets and mouse models (*16, 17*). Interferon has been used clinically when antiviral drugs are unavailable, e.g. against filoviruses (*18*). Replication of SARSr-CoV is sensitive to interferon treatments, as shown in our previous work (*16*); **ii)** Boosting bat IFN by blocking bat-specific IFN negative regulators. Uniquely, bat IFNA is naturally constitutively expressed but cannot be induced to a high level (*5*), indicating a negative regulatory factor in the bat interferon production pathway. We will use CRISPRi to identify the negative regulator and then screen for compounds targeting this gene; **iii)** Activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7-dependent pathways. Our work showing that mutant bat STING restores antiviral functionality suggests these pathways are important in bat-viral coexistence (*4*). By identifying small molecules to directly activate downstream of STING, we will activate bat interferon and promote viral clearance. A similar strategy will be applied to ssRNA-TLR7-dependent pathways; **iv)** Activating functional bat IFN production pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been demonstrated in a mouse model for SARS-CoV, IAV and HBV (*17, 19*); **v)** Inoculating crude coronavirus fragments to upregulate innate immune responses to specific CoVs – a partial step towards the targeted immune priming work below.

Prof. Ralph Baric (UNC) will lead the immune priming work. He will develop recombinant chimeric spike-proteins (*20*) from our known SARSr-CoVs, and those we characterize during project DEFUSE. The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain (*21*). In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles (*11, 22-25*). We will test these in controlled lab conditions, taking the best candidate forward for testing in the field. We have built recombinant spike glycoproteins harboring structurally

defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broadscale immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, potentially reducing disease risk (and transmission risk to people) in these animals for longer periods (*26, 27*).

The immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established the only experimental breeding colony of cave bats (*Eonycteris spelaea*) in SE Asia. This genus is evolutionarily closely related to *Rhinolophus* spp. (the hosts of SARSr-CoVs), so we have confidence that results will be transferable. Our initial proof-of-concept tests will be in this experimental colony, extended to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with *Rhinolophus* sp. bats in the BSL-4 facility at CSIRO, AAHL (L.Wang, unpublished results).

Finally, work on a delivery method for our immune boosting and priming molecules will be overseen by Dr. Tonie Rocke at the USGS, National Wildlife Health Center who has previously developed animal vaccines through to licensure (*28*). Using locally acquired insectivorous bats (*29, 30*) we will assess delivery vehicles and methods including: 1) transdermally applied nanoparticles; 2) series of sticky edible gels that bats will groom from themselves and each other; 3) aerosolization via sprayers that could be used in cave settings; 4) automated sprays triggered by timers and movement detectors at critical cave entry points; 5) sprays delivered by remote controlled drone. We have already used simple gels to vaccinate bats against rabies in the lab (*29*), and hand delivered these containing biomarkers to vampire bats in Peru and Mexico to show they are readily consumed and transferred among bats. In our bat colony, we will trial delivery vehicles using the biomarker rhodamine B (which marks hair and whiskers upon consumption) to assess uptake. The most optimal approaches will then be tested on wild bats in our three cave sites in Yunnan Province with the most successful immunomodulators from TA2. Fieldwork will be conducted under the auspices of Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance). A small number of bats will be captured and assayed for viral load and immune function after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has had unique access to these sites for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for experimental trials from the Provincial Forestry Department. We expect to be successful, as we have worked with the Forestry Department collaboratively for 10 years, with support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife. EHA has a proven track record of rapidly obtaining IACUC and DoD ACURO approval for bat research.

E. Capabilities:

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research organization focused on emerging zoonotic diseases. The PI, Dr. Peter Daszak, has 25+ years' experience managing lab, field and modeling research projects on

emerging zoonoses. Dr. Daszak will commit 3 months annually to oversee and coordinate all project activities, and lead modeling and analytic work for TA1. Dr. Billy Karesh has 40+ years' experience leading zoonotic and wildlife disease projects, and will commit 1 month annually to manage partnership activities and outreach. Dr. Jon Epstein, with 15 years' experience working emerging bat zoonoses will coordinate animal trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project. Support staff include field surveillance teams, modeling analysts, and consultants based in Yunnan Province, China, to oversee field trials. The EHA team has worked extensively with all other collaborators: Prof. Wang (15+ years); Dr. Shi (15+ years); Prof. Baric (5+ years) and Dr. Rocke (15+ years).

Subcontracts: #1 to Prof. Ralph Baric, UNC, to oversee reverse engineering of SARSr-CoVs, BSL-3 humanized mouse experimental infections, design and testing of immune priming treatments based on recombinant spike proteins. Assisted by senior personnel Dr. Tim Sheahan, Dr. Amy Sims, and support staff; **#2** to Prof. Linfa Wang, Duke NUS, to oversee the immune boosting approach, captive bat experiments, and analyze immunological and virological responses to immune boosting treatments; **#3** to Dr. Zhengli Shi, Wuhan Institute of Virology, to conduct PCR testing, viral discovery and isolation from bat samples collected in China, spike protein binding assays, and some humanized mouse work, as well as experimental trials on *Rhinolophus* bats. Her team will include Dr. Peng Zhou and support staff; **#4** to Dr. Tonie Rocke, USGS National Wildlife Health Center, to refine delivery mechanisms for both immune boosting and immune priming treatments. With a research technician, Dr. Rocke will use a captive colony of bats at NWHC for initial trials, and oversee cave experiments in China.

F. *Links to published papers, resume of two key performers*

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based research organization focused on emerging zoonotic diseases. His >300 scientific papers include the first global map of EID hotspots (*31, 32*), estimates of unknown viral diversity (*33*), predictive models of virus-host relationships (*7*), and evidence of the bat origin of SARS-CoV (*34, 35*) and other emerging viruses (*36-39*). He is Chair of the NASEM Forum on Microbial Threats, and is a member of the Executive Committee and the EHA institutional lead for the $130 million USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Dept. of Epidemiology and Dept. of Microbiology & Immunology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, cross species transmission and pathogenesis. His group has developed a platform strategy to access the potential "pre-epidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (*8-13*).

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9. V. D. Menachery *et al.*, MERS-CoV and H5N1 influenza virus antagonize antigen presentation by altering the epigenetic landscape. *Proc Natl Acad Sci U S A* **115**, E1012-E1021 (2018).
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RE: Final draft DARPA abstract

Wang Linfa <linfa.wang@duke-nus.edu.sg>

Tue 2/13/2018 7:59 AM

To: Peter Daszak <daszak@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
Cc: Jonathon Musser <musser@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Rocke, Tonie E <trocke@usgs.gov>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>

Thanks Peter.

Dear Luke,
It will be good if you can circulate the final/submitted version to us all as well.

Fingers crossed.

LF

Linfa (Lin-Fa) WANG, PhD FTSE
Professor & Director
Programme in Emerging Infectious Disease
Duke-NUS Medical School,
8 College Road, Singapore 169857
Tel: +65 6516 8397

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Tuesday, 13 February, 2018 9:21 PM
To: Noam Ross; Luke Hamel
Cc: Jonathon Musser; William B. Karesh; Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Rocke, Tonie; 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby
Subject: RE: Final draft DARPA abstract
Importance: High

Here's the Abstract version 4 for your files.

Luke – please do the following ASAP today:

1. Fix the references – remove the endnote links, and use the format: "(*1,2*)", but making these blue, and an embedded hyperlink to the NCBI paper
2. Insert the corrected figure
3. Make sure we have the right format throughout – including grant number etc. at the top
4. Insert the timeline and budget
5. Send it in!

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4473
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Noam Ross [mailto:ross@ecohealthalliance.org]
Sent: Tuesday, February 13, 2018 6:22 AM
To: Peter Daszak; Luke Hamel
Cc: Jonathon Musser; William B. Karesh; Ralph Baric (rbaric@email.unc.edu); wang linfa; Rocke, Tonie; 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby
Subject: Re: Final draft DARPA abstract

Added just a couple of small edits on modeling phrasing, done on top of Tonie's edits.

On Tue, Feb 13, 2018 at 2:47 AM Rocke, Tonie <trocke@usgs.gov> wrote:

> Just minor edits. Looks good. -Tonie
>
> On Mon, Feb 12, 2018 at 11:23 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:
> Luke, attached is the DARPA abstract.
>
> First thing tomorrow, can you go through the references, and create links to the papers on NCBI. Just turn each reference citation: "(*1*)" for example, into a live link to the paper on NCBI. We only have 2 spare lines, so no room to turn each of these into a PMC numbered ref, as Ralph did for his, but please make sure the citation in parentheses is blue, so it's clear it's a live link on the final pdf.
>
> Ralph, Lina, Peng, Zhengli, Tonie – please give this a quick read to make sure I've not said anything completely wrong. I've had to reduce the text a lot to hit the page limit, but I still think it's a great proposal.
>
> I'll finish off the exec summary slide and <500 wd abstract now.
>
> Cheers,
>
> Peter
>
> **Peter Daszak**
> *President*
>
> EcoHealth Alliance

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--
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--
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Re: Final DARPA Exec Summary slide

Luke Hamel <hamel@ecohealthalliance.org>

Tue 2/13/2018 8:19 AM

To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: Jonathon Musser <musser@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; wang linfa <linfa.wang@duke-nus.edu.sg>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

Hi Peter,

Jonathon and I will be sure to do a final check on everything. Also, I'm not seeing the final summary slide. Could you please reattach it?

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
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On Tue, Feb 13, 2018 at 1:03 AM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

> Hi Luke and Jonathon.
>
> Here's the edited, final summary slide.
>
> Please insert the re-drawn figure, and the budget numbers from Aleksei tomorrow before you submit
>
> NB –there are other things to check on the Abstract, including budget numbers, timeline, the references, and then please do a final check on the compliance with DARPA instructions for all!

Cheers,

Peter

Peter Daszak

President

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From: Peter Daszak
Sent: Tuesday, February 13, 2018 12:23 AM
To: Luke Hamel (hamel@ecohealthalliance.org); Jonathon Musser
Cc: William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); wang linfa; 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura (chmura@ecohealthalliance.org); 'Anna Willoughby (willoughby@ecohealthalliance.org)'; Rocke, Tonie
Subject: Final draft DARPA abstract
Importance: High

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Ralph, Lina, Peng, Zhengli, Tonie – please give this a quick read to make sure I've not said anything completely wrong. I've had to reduce the text a lot to hit the page limit, but I still think it's a great proposal.

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Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4473

www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

Re: Final draft DARPA abstract

Luke Hamel <hamel@ecohealthalliance.org>

Tue 2/13/2018 8:23 AM

To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: Noam Ross <ross@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; wang linfa <linfa.wang@duke-nus.edu.sg>; Rocke, Tonie E <trocke@usgs.gov>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>

Absolutely. I'll start on these items right away, Peter.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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Tue 2/13/2018 8:26 AM

To: Wang Linfa <linfa.wang@duke-nus.edu.sg>
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> LF

Linfa (Lin-Fa) WANG, PhD FTSE

Professor & Director

Programme in Emerging Infectious Disease

Duke-NUS Medical School,

8 College Road, Singapore 169857

Tel: +65 6516 8397

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Important: This email is confidential and may be privileged. If you are not the intended recipient, please delete it and notify us immediately; you should not copy or use it for any purpose, nor disclose its contents to any other person. Thank you.

RE: Final DARPA Exec Summary slide

Peter Daszak <daszak@ecohealthalliance.org>

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Hi Peter,

Jonathon and I will be sure to do a final check on everything. Also, I'm not seeing the final summary slide. Could you please reattach it?

Best,

Luke Hamel
Program Assistant

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On Tue, Feb 13, 2018 at 1:03 AM, Peter Daszak <daszak@ecohealthalliance.org> wrote:
Hi Luke and Jonathon.

Here's the edited, final summary slide.

Please insert the re-drawn figure, and the budget numbers from Aleksei tomorrow before you submit

NB –there are other things to check on the Abstract, including budget numbers, timeline, the references, and then please do a final check on the compliance with DARPA instructions for all!

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Peter

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President

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Executive Summary: Proposal Title

EcoHealth Alliance; Dr. Peter Daszak

CONCEPT

Provide graphic.

APPROACH

Describe new ideas.

IMPACT

Describe need and problem being addressed.

Describe goal.

CONTEXT

Describe existing approaches; compare to state of the art.

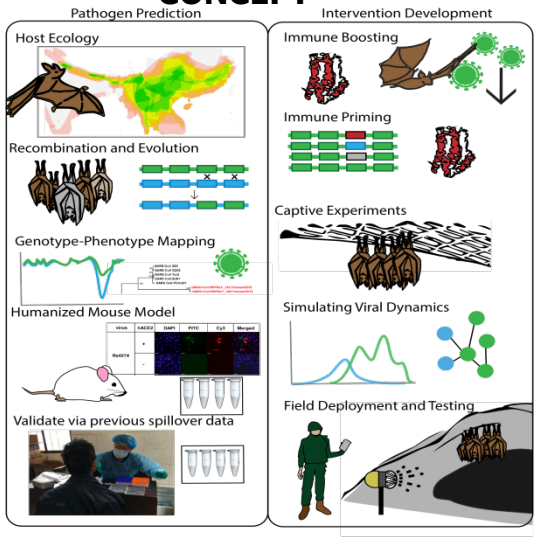
	Phase I	Phase II	Total
Proposed	$-	$-	$-

☒Human Use/ ☒ Animal Use

Executive Summary: DEFUSE

EcoHealth Alliance; Dr. Peter Daszak

CONCEPT



APPROACH

Pathogen Prediction:

- Host-Pathogen Ecology: Develop host-pathogen ecological niche models based on unique bat and viral data, to estimate likelihood of spillover of SARS-related CoVs into human populations. Doing so will enhance predictive ability of models beyond sampling sites in China to cover all Asia.
- Mobile Application: Create 'Reservoirs Near Me' mobile application, to assess background risk of disease spillover for any site across Southeast Asia.
- Binding and Humanized mouse asssays: Utilize team's unique collaboration between world-class modelers and virologists with CoV expertise to conduct spike protein-based binding and humanized mice experiments.
    - Use results to test machine-learning genotype-to-phenotype model predictions of viral spillover risk.
- Genotype-Phenotype Models: Develop models to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Inputs to include: Diversity of bat spike proteins, prevalence of recombinant CoVs, and flow of genes within each bat cave via bat movement and migration.
- Validation with Human Sera: Analyze new and existing human serum samples to validate model outputs. Given frequent SARSr-CoV spillover events into local human populations, this can be done to a degree not possible in systems where spillover events are rare.

Intervention Development: (2 parallel approaches)

- (1) Broadscale Immune Boosting Strategy: Inoculate bats with immune modulators to upregulate innate immune response and downregulate viral replication, transiently reducing risk of viral shedding and spillover.
- (2) Targeted Immune Priming Strategy: Inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance innate immune response against specific, high-risk viruses.
- Viral Dynamics: Develop stochastic simulation models to estimate the frequency, efficacy, and population coverage required for intervention approaches to effectively suppress the viral population.
- Field Deployment and Testing: Ulilze team's expertise in wildlife vaccine delivery to assess and deploy effective molecule delivery methods, including: automated aerosolization technology that inoculates bats as they leave cave roost; remote controlled drone technology; transdermal nanoparticle application; and application of edible, adhesive gels that bats ingest when grooming fur of self and others.

IMPACT

- Recent and ongoing security concerns within South and SE Asia make the region a likely deployment site for US warfighters. Troops deployed to the region face increased disease risk from SARS and related bat viruses, as bats shed these pathogens through urine and feces while foraging over large areas at night.
- Our work in Yunnan Province, China has shown that (1) SARSr-CoVs are capable of producing SARS-like illness in humanized mice that are not affected by monoclonal or vaccine treatment, and (2) that spillover into local human populations is frequent. With no available vaccine or alternative method to counter these SARS-related viruses, US defense forces and national security are placed at risk.
- Our goal is to "DEFUSE" the potential for emergence of novel bat-origin high zoonotic risk SARSr-CoVs in Southeast Asia. In doing so, we will not only safeguard the US warfighter, but also reduce SARSr-CoV exposure for local communities and their livestock, improving food security Global Health Security.
- If successful, our strategy can be adapted to hosts of other bat-origin CoVs (MERS-CoV in the Middle East and other SARS-related pre-pandemic zoonotic strains in Africa, e.g. Nigeria), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, Ebola viruses).

CONTEXT

- No technology currently exists to reduce the risk of exposure to novel bat Coronaviruses.
- Our team has conducted pioneering research on modeling disease emergence, understanding Coronavirus virology, bat immunity, and wildlife vaccine delivery. Our previous work provides proof-of-concept for: (1) predictive 'hotspot' modeling; (2) upregulating bat immune response through the STING IFN pathway, (2) developing recombinant chimeric spike-proteins from SARS and SARSr-CoVs and (3) delivering immunological countermeasures to wildlife (including multiple bat species).
- The DEFUSE approach is broadly effective, scalable, economical and achievable in the allotted time frame. It also poses little environmental risk, and presents no threat to local livestock or human populations.
- While CRISPR-Cas9 gene drives are being considered for many disease research applications, the technique is unlikely to be effective in suppressing viral transmission in bat hosts. Bats are relatively long-lived, highly mobile, and have long inter-generational periods (2-5 years) with low progeny (1-2 pups). Furthermore, gene drive technology could have far-reaching, negative ecological consequences and its effectiveness cannot be evaluated within the defined Period of Performance.

	Phase I	Phase II	Total
Proposed	$-	$-	$-

☒Human Use/ ☒ Animal Use

Re: First (rough) draft of the DARPA abstract - Project DEFUSE

Richgels, Katherine L <krichgels@usgs.gov>

Thu 2/15/2018 9:05 AM

To: Sleeman, Jonathan M <jsleeman@usgs.gov>
Cc: Rocke, Tonie E <trocke@usgs.gov>

Hi Tonie,

I am fully supportive of this. The proposal is risky and exciting, and would align quite nicely with your ongoing bat work.

As you stated yesterday in our branch meeting, I agree that we need to be looking for new outside funding and DARPA is certainly one of those opportunities.

Katie

On Fri, Feb 9, 2018 at 5:42 AM, Sleeman, Jonathan <jsleeman@usgs.gov> wrote:

> Hi Tonie,
>
> If Katie concurs I am supportive. Peter always has crazy ideas. I would also like to talk to you about the vampire bat colony when we are both in the office.
>
> By the way, no one at the NWHC questions the value of the Research Branch. In fact, it is quite the contrary,
>
> Best wishes,
>
> Jonathan
>
> On Thu, Feb 8, 2018 at 8:27 AM, Rocke, Tonie <trocke@usgs.gov> wrote:
>
> > Hi Katie: As I mentioned to you by phone, I have been asked to collaborate on a proposal to DARPA with EcoHealth Alliance. I am forwarding you and Jonathan the first draft I received to keep you in the loop so you know what is going on. There is alot here I need to correct (i.e. we don't have a captive Jamaican fruit bat colony but we are thinking of setting up a vampire bat colony at UW) and I will be working on editing the draft proposal.
> >
> > Also, I have been asked to collaborate on a second proposal by another group (BU emerging infectious disease unit) for the same RFA

that involves morbillivirus and rabies in vampire bats in Latin America, although I haven't seen a draft of that proposal yet.

At this point, I plan to participate in both proposals as there is no restriction in that regard, but let me know soon if you have any questions/concerns. Due date for abstracts is approaching very rapidly - Feb 13. No guarantee of funding of course, but just the fact that we are being asked to collaborate on these proposals should be taken as evidence of the relevance of the NWHC research branch, which seems to be in question lately. -Tonie

---------- Forwarded message ----------
From: **Peter Daszak** <daszak@ecohealthalliance.org>
Date: Wed, Feb 7, 2018 at 8:51 PM
Subject: First (rough) draft of the DARPA abstract - Project DEFUSE
To: "Ralph Baric (rbaric@email.unc.edu)" <rbaric@email.unc.edu>, Wang Linfa <linfa.wang@duke-nus.edu.sg>, "Zhengli Shi (zlshi@wh.iov.cn)" <zlshi@wh.iov.cn>, "William B. Karesh" <karesh@ecohealthalliance.org>, "Rocke, Tonie" <trocke@usgs.gov>
Cc: Luke Hamel <hamel@ecohealthalliance.org>, Jonathon Musser <musser@ecohealthalliance.org>, Anna Willoughby <willoughby@ecohealthalliance.org>, "Kevin Olival, PhD" <olival@ecohealthalliance.org>, Jon Epstein <epstein@ecohealthalliance.org>, Noam Ross <ross@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>, Hongying Li <li@ecohealthalliance.org>

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.

Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4473

www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

--
Jonathan Sleeman, MA, VetMB, Dipl. ACZM, Dipl. ECZM, MRCVS
Center Director
USGS, National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711

Tel: (608) 270 2401
Fax: (608) 270 2415
Email: jsleeman@usgs.gov

> ***The USGS National Wildlife Health Center's mission is to safeguard wildlife and ecosystem health through dynamic partnerships and exceptional science***
>
> ***OIE Collaborating Centre for Research, Diagnosis and Surveillance of Wildlife Pathogens***

--
Katherine L. D. Richgels, Ph.D.
Branch Chief, Applied Wildlife Health Research
Responsible Official, Federal Select Agent Program
USGS National Wildlife Health Center
6006 Schroeder Rd
Madison, WI 53711
(608) 270 - 2450 (office)
(608) 381 - 2492 (cell)
(608) 270 - 2415 (fax)
krichgels@usgs.gov
www.nwhc.usgs.gov

Re: RE: Final DARPA Exec Summary slide

石正丽 <zlshi@wh.iov.cn>

Thu 2/15/2018 8:52 PM

To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; wang linfa <linfa.wang@duke-nus.edu.sg>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

Thanks.

> -----原始邮件-----
> **发件人:**"Peter Daszak" <daszak@ecohealthalliance.org>
> **发送时间:**2018-02-14 00:28:21 (星期三)
> **收件人:** "Luke Hamel" <hamel@ecohealthalliance.org>
> **抄送:** "Jonathon Musser" <musser@ecohealthalliance.org>, "William B. Karesh" <karesh@ecohealthalliance.org>, "Noam Ross" <ross@ecohealthalliance.org>, "Ralph Baric (rbaric@email.unc.edu)" <rbaric@email.unc.edu>, "wang linfa" <linfa.wang@duke-nus.edu.sg>, "周鹏 (peng.zhou@wh.iov.cn)" <peng.zhou@wh.iov.cn>, "Zhengli Shi (zlshi@wh.iov.cn)" <zlshi@wh.iov.cn>, "Alison Andre" <andre@ecohealthalliance.org>, "Aleksei Chmura" <chmura@ecohealthalliance.org>, "Anna Willoughby" <willoughby@ecohealthalliance.org>, "Rocke, Tonie" <trocke@usgs.gov>
> **主题:** RE: Final DARPA Exec Summary slide
>
> Cheers,
>
> Peter
>
> **Peter Daszak**

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4473

www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Luke Hamel [mailto:hamel@ecohealthalliance.org]
Sent: Tuesday, February 13, 2018 9:19 AM
To: Peter Daszak
Cc: Jonathon Musser; William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); wang linfa; 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby; Rocke, Tonie
Subject: Re: Final DARPA Exec Summary slide

Hi Peter,

Jonathon and I will be sure to do a final check on everything. Also, I'm not seeing the final summary slide. Could you please reattach it?

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)

(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Tue, Feb 13, 2018 at 1:03 AM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

Hi Luke and Jonathon.

Here's the edited, final summary slide.

Please insert the re-drawn figure, and the budget numbers from Aleksei tomorrow before you submit

NB –there are other things to check on the Abstract, including budget numbers, timeline, the references, and then please do a final check on the compliance with DARPA instructions for all!

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

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From: Peter Daszak
Sent: Tuesday, February 13, 2018 12:23 AM
To: Luke Hamel (hamel@ecohealthalliance.org); Jonathon Musser
Cc: William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); wang linfa; 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura (chmura@ecohealthalliance.org); 'Anna Willoughby (willoughby@ecohealthalliance.org)'; Rocke, Tonie
Subject: Final draft DARPA abstract
Importance: High

Luke, attached is the DARPA abstract.

First thing tomorrow, can you go through the references, and create links to the papers on NCBI. Just turn each reference citation: "(*1*)" for example, into a live link to the paper on NCBI. We only have 2 spare lines, so no room to turn each of these into a PMC numbered ref, as Ralph did for his, but please make sure the citation in parentheses is blue, so it's clear it's a live link on the final pdf.

Ralph, Lina, Peng, Zhengli, Tonie – please give this a quick read to make sure I've not said anything completely wrong. I've had to reduce the text a lot to hit the page limit, but I still think it's a great proposal.

I'll finish off the exec summary slide and <500 wd abstract now.

Cheers,

Peter

Peter Daszak

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RE: Final draft DARPA abstract, and next steps...

Peter Daszak <daszak@ecohealthalliance.org>

Tue 2/20/2018 3:47 PM

To: Wang Linfa <linfa.wang@duke-nus.edu.sg>; Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

Dear All,

Apologies – forgot to send you the final versions of the abstract and the executive summary slide for your records…

Re. the total budget – please don't view that as final – they're planning to give out $40 million over the 3.5 years total to anything from 1 to 6 projects. Given that there were probably 3 or 4 other viable teams in the room at the proposer's conference, I think that $14.8 million is probably the maximum they would give any project, and it's more likely that they'll fund three or four at 8 million and two or three smaller projects. They'll hopefully give some clearer guidance as we move towards a full proposal.

The due date for proposals is March 27th, 4pm EST (NY time). That means we need to start honing in on our plans for the full proposal. I'm meeting with our team here on Thursday to start working on the next steps, and it would be good to have calls with all of you this week to start refining the ideas and building out the proposal. Luke will follow up with times that work for phone calls.

Thanks again for your openness and clever ideas, and I look forward to working with you all to get the best possible full proposal submitted on time!!!

Cheers,

Peter

Peter Daszak
President

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From: Peter Daszak
Sent: Tuesday, February 13, 2018 8:15 AM
To: 'Wang Linfa'; Luke Hamel; Jonathon Musser
Cc: William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby; Rocke, Tonie
Subject: RE: Final draft DARPA abstract

Good point Linfa – changed to 'treatment' or 'application' throughout.

Cheers,

Peter

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From: Wang Linfa [mailto:linfa.wang@duke-nus.edu.sg]
Sent: Tuesday, February 13, 2018 1:10 AM
To: Peter Daszak; Luke Hamel; Jonathon Musser
Cc: William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby; Rocke, Tonie
Subject: RE: Final draft DARPA abstract

Hi Peer,

It actually reads well now and I hope the selection committee will "buy in" our brave ideas!

Nothing to add a or change, other than an English question: we used "dampened immunity of bats" in most places, but you use the expression of "damping innate immunity pathways" on page 1. Is there a difference between "damping" and "dampening"?

Thanks

LF

Linfa (Lin-Fa) Wang, PhD FTSE

Professor & Director
Programme in Emerging Infectious Diseases
Duke-NUS Medical School
8 College Road, Singapore 169875
Tel: +65 65168397

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Tuesday, 13 February 2018 1:23 PM
To: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
Subject: Final draft DARPA abstract
Importance: High

Luke, attached is the DARPA abstract.

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Ralph, Lina, Peng, Zhengli, Tonie – please give this a quick read to make sure I've not said anything completely wrong. I've had to reduce the text a lot to hit the page limit, but I still think it's a great proposal.

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Cheers,

Peter

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President

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ABSTRACT - COVER PAGE

TITLE: PROJECT DEFUSE

FUNDER AND BAA: DARPA – PREEMPT (HR001118S0017)

TECHNICAL & LEAD POINT OF CONTACT:

DR. PETER DASZAK
ECOHEALTH ALLIANCE
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NEW YORK, NY 10001
Phone: +1.212.380.4474
Fax: +1.212.380.4465
(e) daszak@ecohealthalliance.org

ADMINISTRATIVE POINT OF CONTACT:

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NEW YORK, NY 10001
Phone: +(b) (6)
Fax: +1.212.380.4465
(e) hamel@ecohealthalliance.org

LEAD ORGANIZATION: EcoHealth Alliance

PRIMARY SUBCONTRACTORS:

Duke-National University Singapore Medical School
National Wildlife Health Center, United States Geological Survey
University of North Carolina, School of Medicine
Wuhan Institute of Virology

ESTIMATED COST: $14,799,998.00

PROJECT DURATION: 3.5 Years

C. Goals and Impact:

1. What is the proposed work attempting to accomplish or do?

We will **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses**. We envisage a scenario whereby the US warfighter is deployed to a security hotspot in SE Asia. As planners choose sites for the mission, they will use an app we will design based on machine-learning models of the ecological and evolutionary potential of bat viruses to spillover. This will allow rapid assessment of the background risk of a site harboring dangerous zoonotic viruses. If there is no alternative site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release **broadscale immune boosting molecules** and chimeric polyvalent spike protein **targeted immune priming treatments** to upregulate the naturally damped innate immune response of bats, and lower viral shedding from bats at the site for a few weeks or months, allowing
our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

Other than PPE, there is no available current technology to reduce the risk of exposure to novel coronaviruses from bats. Models of bat host capacity to harbor viruses, of ecological and environmental drivers of their emergence, and of the evolutionary potential of different strains to spillover are rudimentary. No vaccines or therapeutics exist for SARSr-CoVs, and exposure mitigation strategies are non-existent. SARSr-CoVs are endemic in Asian, African *(1)*, and European bats *(2)* that roost in caves but forage widely at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARSr-CoVs into people in China and have isolated strains capable of producing SARS-like illness in humanized mice that don't respond to antibody treatment or vaccination. These viruses are a clear-and-present danger to our military and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

Our group leads the world in predictive models of viral emergence. We will build on our machine-learning models of spillover hotspots, host-pathogen ecological niches and genotype-phenotype mapping by incorporating unique datasets to validate and refine hotspot risk maps of viral emergence in SE Asia and beyond. Our group has shown that bats coexist with lethal viruses by damping innate immunity pathways, likely as an evolutionary adaptation to flight. We will use this insight to design strategies, like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists, to upregulate bat immunity in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover **(broadscale immune boosting strategy)**. We will complement this by treating bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against specific, high-risk viruses **(targeted immune priming strategy)**, especially when their immune response is boosted as above. We will design novel methods to deliver these applications remotely to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?
Modeling: Previous models have suffered from a lack of data to validate them. We have access to unique datasets that will allow us to validate our approach, including biodiversity surveys of bat caves across S. China, 10+ years of bat viral testing data in China, and 10 other countries (from NIH-NIAID and USAID EPT PREDICT work). Uniquely, we will validate our models of viral evolution/spillover risk using human serology (based on LIPS assays) in local populations that have high (~3%) seroprevalence to bat SARSr-CoVs. *Identifying Immune boosting and priming treatments:* Some of our approaches are novel and challenging (e.g. using CRISPRi to find the negative regulator for bat interferon production), and others are unproven in bats (e.g. Poly IC). We will begin all immune boosting and priming experiments at the beginning of the project, running them simultaneously and competitively, so that we field trial only the most efficient, cost-effective and scalable approaches.

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. Potential deployment to regions where SARSr-CoVs exist is high – countries include security hotspots in Asia (e.g. Myanmar, Bangladesh, Pakistan, Korea, Vietnam), Africa and Eastern Europe. The ability to decontaminate and defuse these viruses may prevent potentially devastating illness. These technologies could be adapted to hosts of other bat-origin CoVs (e.g. MERS-CoV, SADS-CoV) and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV), with benefits to livestock production, food security and global public health.

6. How much will it cost and how long will it take?

	Phase I (24 months)	Phase II (18 months)	Total
Proposed	$8,414,104	$6,385,894	$14,799,998

D. Technical Plan:
Overview
The SARSr-CoV-bat system, and immune modulation focus: Our group's 15 yrs work on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014 & WIV-1). We have shown they bind and replicate efficiently in primary human lung airway cells and that chimeras with SARSr-CoV spike proteins in a SARS-CoV backbone cause SARS-like illness in humanized mice, with clinical signs that are not reduced by SARS monoclonal therapy or vaccination. We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV *(7,8,9)*. We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**. Our work on bat immunology suggests that bats' unique flying ability has led to downregulated innate immune genes, and their ability to coexist with viruses such as

SARSr-CoVs, henipa- and filoviruses that are lethal in many other mammals (3). We have identified bat-specific constitutively expressed bat interferon, a dampened STING-interferon production pathway (4, 5), and have identified a series of other innate immunity factors that are dampened in bats (6).

Our bat-CoV system has significant advantages for experimentation and intervention. Firstly, these viruses are fecal-orally transmitted within bat populations, so sampling can be achieved from fresh fecal pellet collection. They are BSL-3, not -4, agents, so that experimental manipulation and infection is simpler. They have frequent spillover events, making it possible to validate predictive models of spillover by sampling people. They are diverse, with frequent recombination and different strains exhibiting differential host cell binding and spillover potential. Finally, we have identified SARSr-CoV strains in a single cave in Yunnan that harbor all of the epidemic SARS-CoV genes. This specific bat population harbors an ideal evolutionary soup that could produce new human strains by high frequency RNA recombination, and thus, it presents a perfect target for next generation, technology-forward intervention strategies.

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team, led by Drs. Daszak, Ross, Olival, EHA, will build ecological niche models of environmental and ecological correlates and traits of cave bat communities to predict species composition of bat caves across Southern China, South and SE Asia. We will then use a series of datasets we have built to produce host-virus risk models for the region. These include our unique database of bat host-viral relationships (7); biological inventory data on all bat caves in Southern China; and modeled species distribution data for all bats. We will parameterize the model with data from three cave sites in Yunnan, China (one with high-risk SARSr-CoVs, two other control/comparison sites), including: radio- and GPS-telemetry to identify home range and additional roost sites for each bat species; inventory of bat population density, distribution and segregation and their daily, weekly and seasonal changes; viral prevalence and individual viral load; shedding of low- and high-risk SARSr-CoV strains among bat species, age classes, genders; and telemetry and mark-recapture data to assess metapopulation structure and inter-cave connectivity. We will test and validate model predictions of a cave's viral spillover potential with data from prior PREDICT sampling in 7 other Asian countries. At the end of Yr 1, we will produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens in a region. **The 'high-risk bats near me' app** will be updated real-time with surveillance data (e.g. field-deployable iPhone and android compatible echolocation data) from our project and others, to ground-truth and fine-tune its predictive capacity.

The Wuhan Institute of Virology team will test bat fecal, oral, blood and urogenital samples for SARSr-CoVs. We will correlate viral load data from these samples with fresh fecal pellets from individuals and from tarps laid on cave floors. We will rapidly move to fecal pellet assays to reduce roost disturbance. SARSr-CoV spike proteins will be sequenced, analyzed phylogenetically for recombination events, and high-risk viruses (spike proteins close to SARS-CoV) characterized and isolated. The UNC team will reverse-engineer spike proteins to conduct

binding assays to human ACE2 (the SARS-CoV receptor). They will culture SARS-like bat coronaviruses to distinguish high-risk strains that can replicate in primary human cells and low risk strains that require exogenous enhancers. Viral spike glycoproteins that bind receptors will be inserted into SARS-CoV backbones, inoculated into human cells and humanized mice to assess capacity to cause SARS-like disease, and to be blocked by monoclonal therapies, the nucleoside analogue inhibitor GS-5734*(8)* or vaccines against SARS-CoV *(8,9,10,11,12,13).*

The EHA modeling team will use these data to **build models of risk of viral evolution and spillover**. These genotype-to-phenotype machine-learning models will predict viral ability to infect host cells based on genetic traits and results of receptor binding and mouse infection assays. Using data on diversity of spike proteins, recombinant CoVs, and flow of genes within each bat cave via bat movement and migration, we will estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Finally, virus-host relationship and bat home range data will be used to estimate spillover potential - extending models well beyond our field sites. We will then **validate model predictions of viral spillover risk** by **1)** conducting spike protein-based binding and cell culture experiments, and **2)** identifying spillover strains in people near our bat cave sites. Our preliminary work on this shows ~3% seroprevalence to SARSr-CoVs, using a specific ELISA *(14).* We will design LIPS assays to the specific high- and low- zoonotic-risk SARSr-CoVs identified in this project as we have done previously *(15).* We will use banked and newly collected human sera from these populations to test for presence of antibodies to the high- and low-risk SARSr-CoVs identified by our modeling. We will then **model optimal strategies to maximize treatment efficacy** for TA2, using stochastic simulation modeling informed by field and experimental data to characterize viral circulation dynamics in bats. We will estimate frequency and population coverage required for our intervention approaches to suppress viral spillover. We will determine the seasons, locations within a cave, and delivery methods (spray, swab, or automated cave mouth or drone) that will be most effective. Finally we will determine the time period treatment will be effective for, until re-colonization or evolution leads to return of a high-risk SARSr-CoV.

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

We will evaluate two approaches to defuse SARS-related CoV spillover potential**: 1) Broadscale Immune Boosting:** using the unique immune damping in bats that our group has discovered, we will apply immune modulators like bat interferon to live bats, to up-regulate their naïve immunity and then assess their ability to suppress viral replication and shedding; **2) Targeted Immune Priming:** building on preliminary development of polyvalent chimeric recombinant SARSr-CoV spike proteins, we will conduct application trials with live bats to assess suppression of replication and shedding of a broad range of dangerous SARS-related CoVs.

Both lines of work will begin in Yr 1 and run parallel. Prof. Linfa Wang (Duke-NUS) will lead the immune boosting work, building on his pioneering work on bat immunity *(3)* which shows that the long-term coexistence of bats and their viruses has led to equilibrium between viral replication and host immunity. This is likely due to down-regulation of their innate immune system as a fitness cost of flight *(3)*. The weakened functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may allow bats to maintain an

effective, but not over-response to viruses (*4*). A similar finding was observed for bat IFNA, which is less abundant but constitutively expressed without stimulation (*5*). Given high native SARSr-CoV load in bats, we aim to boost bat innate immunity through the IFN pathway, break the host-virus equilibrium to suppress bat SARSr-CoV replication and shedding.

We will trial the following, concurrently and competitively, for efficiency, cost and scalability: **i)** Universal bat interferon. Aerosol spraying or intranasal application of IFN or other small molecules reduces viral loads in humans, ferrets and mouse models (*16, 17*). Interferon has been used clinically when antiviral drugs are unavailable, e.g. against filoviruses (*18*). Replication of SARSr-CoV is sensitive to interferon treatments, as shown in our previous work (*16*); **ii)** Boosting bat IFN by blocking bat-specific IFN negative regulators. Uniquely, bat IFNA is naturally constitutively expressed but cannot be induced to a high level (*5*), indicating a negative regulatory factor in the bat interferon production pathway. We will use CRISPRi to identify the negative regulator and then screen for compounds targeting this gene; **iii)** Activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7-dependent pathways. Our work showing that mutant bat STING restores antiviral functionality suggests these pathways are important in bat-viral coexistence (*4*). By identifying small molecules to directly activate downstream of STING, we will activate bat interferon and promote viral clearance. A similar strategy will be applied to ssRNA-TLR7-dependent pathways; **iv)** Activating functional bat IFN production pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been demonstrated in a mouse model for SARS-CoV, IAV and HBV (*17, 19*); **v)** Inoculating crude coronavirus fragments to upregulate innate immune responses to specific CoVs – a partial step towards the targeted immune priming work below.

Prof. Ralph Baric (UNC) will lead the immune priming work. He will develop recombinant chimeric spike-proteins (*20*) from our known SARSr-CoVs, and those we characterize during project DEFUSE. The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain (*21*). In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles (*11, 22,23,24,25*). We will test these in controlled lab conditions, taking the best candidate forward for testing in the field. We have built recombinant spike glycoproteins harboring structurally defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broad scale immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, potentially reducing disease risk (and transmission risk to people) in these animals for longer periods (*26, 27*).

The immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established the only experimental breeding colony of cave bats (*Eonycteris spelaea*) in SE Asia. This genus is evolutionarily related to *Rhinolophus* spp. (the hosts of SARSr-CoVs), so we have confidence that results will be transferable. Our initial proof-of-concept tests will be in this experimental colony, extended to a small group of wild-caught *Rhinolophus*

sinicus bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with *Rhinolophus* sp. bats in the BSL-4 facility at CSIRO, AAHL (L.Wang, unpublished results).

Finally, work on a delivery method for our immune boosting and priming molecules will be overseen by Dr. Tonie Rocke at the USGS, National Wildlife Health Center who has previously developed animal vaccines through to licensure *(28)*. Using locally acquired insectivorous bats *(29, 30)* we will assess delivery vehicles and methods including: 1) transdermally applied nanoparticles; 2) series of sticky edible gels that bats will groom from themselves and each other; 3) aerosolization via sprayers that could be used in cave settings; 4) automated sprays triggered by timers and movement detectors at critical cave entry points, and 5) sprays delivered by remote controlled drone. We have already used simple gels to vaccinate bats against rabies in the lab *(29)*, and hand delivered these containing biomarkers to vampire bats in Peru and Mexico to show they are readily consumed and transferred among bats. In our bat colony, we will trial delivery vehicles using the biomarker rhodamine B (which marks hair and whiskers upon consumption) to assess uptake. The most optimal approaches will then be tested on wild bats in our three cave sites in Yunnan Province with the most successful immunomodulators from TA2. Fieldwork will be conducted under the auspices of Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance). A small number of bats will be captured and assayed for viral load and immune function after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has had unique access to these sites for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for experimental trials from the Provincial Forestry Department. We expect to be successful, as we have worked with the Forestry Department collaboratively for 10 years, with support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife. EHA has a proven track record of rapidly obtaining IACUC and DoD ACURO approval for bat research.

E. Capabilities:

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research organization focused on emerging zoonotic diseases. The PI, Dr. Peter Daszak, has 25+ years' experience managing lab, field and modeling research projects on emerging zoonoses. Dr. Daszak will commit 3 months annually to oversee and coordinate all project activities, and lead modeling and analytic work for TA1. Dr. Billy Karesh has 40+ years' experience leading zoonotic and wildlife disease projects, and will commit 1 month annually to manage partnership activities and outreach. Dr. Jon Epstein, with 15 years' experience working emerging bat zoonoses will coordinate animal trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project. Support staff include field surveillance teams, modeling analysts, and consultants based in Yunnan Province, China, to oversee field trials. The EHA team has worked extensively with all other collaborators: Prof. Wang (15+ years); Dr. Shi (15+ years); Prof. Baric (5+ years) and Dr. Rocke (15+ years).

Subcontracts: #1 to Prof. Ralph Baric, UNC, to oversee reverse engineering of SARSr-CoVs, BSL-3 humanized mouse experimental infections, design and testing of immune priming treatments based on recombinant spike proteins. Assisted by senior personnel Dr. Tim

Sheahan, Dr. Amy Sims, and support staff; **#2** to Prof. Linfa Wang, Duke NUS, to oversee the immune boosting approach, captive bat experiments, and analyze immunological and virological responses to immune boosting treatments; **#3** to Dr. Zhengli Shi, Wuhan Institute of Virology, to conduct PCR testing, viral discovery and isolation from bat samples collected in China, spike protein binding assays, and some humanized mouse work, as well as experimental trials on *Rhinolophus* bats. Her team will include Dr. Peng Zhou and support staff; **#4** to Dr. Tonie Rocke, USGS National Wildlife Health Center, to refine delivery mechanisms for both immune boosting and immune priming treatments. With a research technician, Dr. Rocke will use a captive colony of bats at NWHC for initial trials, and oversee cave experiments in China.

F. *Links to published papers, resume of two key performers*

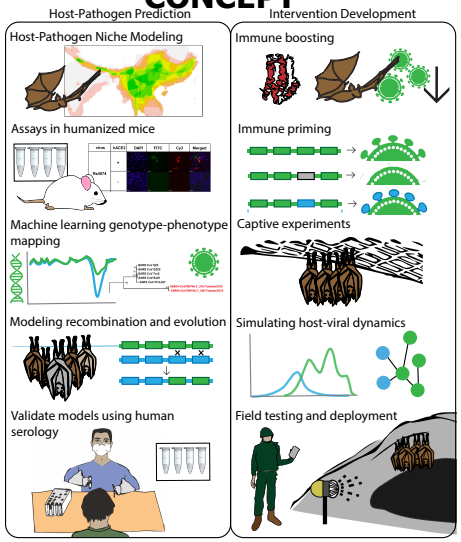
Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based research organization focused on emerging zoonotic diseases. His >300 scientific papers include the first global map of EID hotspots *(31, 32)*, estimates of unknown viral diversity *(33)*, predictive models of virus-host relationships *(7)*, and evidence of the bat origin of SARS-CoV *(34, 35)* and other emerging viruses *(36,37,38,39)*. He is Chair of the NASEM Forum on Microbial Threats, and is a member of the Executive Committee and the EHA institutional lead for the $130 million USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr. Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Dept. of Epidemiology and Dept. of Microbiology & Immunology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, cross species transmission and pathogenesis. His group has developed a platform strategy to access the potential "pre-epidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (*8,9,10,11,12,13*).

Executive Summary: DEFUSE

EcoHealth Alliance; Dr. Peter Daszak

CONCEPT



APPROACH

Host-Pathogen Prediction:

- Host-Pathogen Ecology: Develop host-pathogen ecological niche models based on unique bat and viral data, to estimate likelihood of spillover of SARS-related CoVs into human populations. Doing so will enhance predictive ability of models beyond sampling sites in China to cover all Asia.
- Mobile Application: Create 'Reservoirs Near Me' mobile application, to assess background risk of disease spillover for any site across Southeast Asia.
- Binding and Humanized mouse assays: Utilize team's unique collaboration between world-class modelers and virologists with CoV expertise to conduct spike protein-based binding and humanized mice experiments.
    - Use results to test machine-learning genotype-to-phenotype model predictions of viral spillover risk.
- Genotype-Phenotype Models: Develop models to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Inputs to include: Diversity of bat spike proteins, prevalence of recombinant CoVs, and flow of genes within each bat cave via bat movement and migration.
- Validation with Human Sera: Analyze new and existing human serum samples to validate model outputs. Given frequent SARSr-CoV spillover events into local human populations, this can be done to a degree not possible in systems where spillover events are rare.

Intervention Development: (2 parallel approaches)

- (1) Broadscale Immune Boosting Strategy: Inoculate bats with immune modulators to upregulate innate immune response and downregulate viral replication, transiently reducing risk of viral shedding and spillover.
- (2) Targeted Immune Priming Strategy: Inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance innate immune response against specific, high-risk viruses.
- Viral Dynamics: Develop stochastic simulation models to estimate the frequency, efficacy, and population coverage required for intervention approaches to effectively suppress the viral population.
- Field Deployment and Testing: Utilize team's expertise in wildlife vaccine delivery to assess and deploy effective molecule delivery methods, including: automated aerosolization technology that inoculates bats as they leave cave roost; remote controlled drone technology; transdermal nanoparticle application; and application of edible, adhesive gels that bats ingest when grooming fur of self and others.

IMPACT

- Recent and ongoing security concerns within South and SE Asia make the region a likely deployment site for US warfighters. Troops deployed to the region face increased disease risk from SARS and related bat viruses, as bats shed these pathogens through urine and feces while foraging over large areas at night.
- Our work in Yunnan Province, China has shown that (1) SARSr-CoVs are capable of producing SARS-like illness in humanized mice that are not affected by monoclonal or vaccine treatment, and (2) that spillover into local human populations is frequent. With no available vaccine or alternative method to counter these SARS-related viruses, US defense forces and national security are placed at risk.
- Our goal is to "DEFUSE" the potential for emergence of novel bat-origin high zoonotic risk SARSr-CoVs in Southeast Asia. In doing so, we will not only safeguard the US warfighter, but also reduce SARSr-CoV exposure for local communities and their livestock, improving food security and Global Health Security.
- If successful, our strategy can be adapted to hosts of other bat-origin CoVs (MERS-CoV in the Middle East and other SARS-related pre-pandemic zoonotic strains in Africa, e.g. Nigeria), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, Ebola viruses).

	Phase I	Phase II	Total
Proposed	$8,414,104	$6,385,894	$14,799,998

☒Human Use/ ☒ Animal Use

CONTEXT

- No technology currently exists to reduce the risk of exposure to novel bat Coronaviruses.
- Our team has conducted pioneering research on modeling disease emergence, understanding Coronavirus virology, bat immunity, and wildlife vaccine delivery. Our previous work provides proof-of-concept for: (1) predictive 'hotspot' modeling; (2) upregulating bat immune response through the STING IFN pathway, (2) developing recombinant chimeric spike-proteins from SARS and SARSr-CoVs and (3) delivering immunological countermeasures to wildlife (including multiple bat species).
- The DEFUSE approach is broadly effective, scalable, economical and achievable in the allotted time frame. It also poses little environmental risk, and presents no threat to local livestock or human populations.
- While CRISPR-Cas9 gene drives are being considered for many disease research applications, the technique is unlikely to be effective in suppressing viral transmission in bat hosts. Bats are relatively long-lived, highly mobile, and have long inter-generational periods (2-5 years) with low progeny (1-2 pups). Furthermore, gene drive technology could have far-reaching, negative ecological consequences and its effectiveness cannot be evaluated within the defined Period of Performance.

Re: RE: Final draft DARPA abstract, and next steps...

石正丽 <zlshi@wh.iov.cn>

Tue 2/20/2018 8:55 PM

To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: Wang Linfa <linfa.wang@duke-nus.edu.sg>; Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

Thanks, fingers crossed.

> -----原始邮件-----
> **发件人:**"Peter Daszak" <daszak@ecohealthalliance.org>
> **发送时间:**2018-02-21 05:47:14 (星期三)
> **收件人:** "Wang Linfa" <linfa.wang@duke-nus.edu.sg>, "Luke Hamel" <hamel@ecohealthalliance.org>, "Jonathon Musser" <musser@ecohealthalliance.org>
> **抄送:** "William B. Karesh" <karesh@ecohealthalliance.org>, "Noam Ross" <ross@ecohealthalliance.org>, "Ralph Baric (rbaric@email.unc.edu)" <rbaric@email.unc.edu>, "周鹏 (peng.zhou@wh.iov.cn)" <peng.zhou@wh.iov.cn>, "Zhengli Shi (zlshi@wh.iov.cn)" <zlshi@wh.iov.cn>, "Alison Andre" <andre@ecohealthalliance.org>, "Aleksei Chmura" <chmura@ecohealthalliance.org>, "Anna Willoughby" <willoughby@ecohealthalliance.org>, "Rocke, Tonie" <trocke@usgs.gov>
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Thanks again for your openness and clever ideas, and I look forward to working with you all to get the best possible full proposal submitted on time!!!

Cheers,

Peter

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EcoHealth Alliance

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Peter Daszak
Sent: Tuesday, February 13, 2018 8:15 AM
To: 'Wang Linfa'; Luke Hamel; Jonathon Musser
Cc: William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby; Rocke, Tonie
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Thanks

LF

Linfa (Lin-Fa) Wang, PhD FTSE

Professor & Director

Programme in Emerging Infectious Diseases

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8 College Road, Singapore 169875

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Sent: Tuesday, 13 February 2018 1:23 PM
To: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
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Subject: Final draft DARPA abstract
Importance: High

Luke, attached is the DARPA abstract.

First thing tomorrow, can you go through the references, and create links to the papers on NCBI. Just turn each reference citation: "(*1*)" for example, into a live link to the paper on NCBI. We only have 2 spare lines, so no room to turn each of these into a PMC numbered ref, as Ralph did for his, but please make sure the citation in parentheses is blue, so it's clear it's a live link on the final pdf.

Ralph, Lina, Peng, Zhengli, Tonie – please give this a quick read to make sure I've not said anything completely wrong. I've had to reduce the text a lot to hit the page limit, but I still think it's a great proposal.

I'll finish off the exec summary slide and <500 wd abstract now.

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Linfa (Lin-Fa) Wang, PhD FTSE
Professor & Director
Programme in Emerging Infectious Diseases
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8 College Road, Singapore 169875
Tel: +65 65168397

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Wednesday, 21 February 2018 at 5:47 AM
To: Wang Linfa <linfa.wang@duke-nus.edu.sg>, Luke Hamel <hamel@ecohealthalliance.org>, Jonathon Musser <musser@ecohealthalliance.org>
Cc: William Karesh <karesh@ecohealthalliance.org>, Noam Ross <ross@ecohealthalliance.org>, Ralph Baric <rbaric@email.unc.edu>, 周鹏 <peng.zhou@wh.iov.cn>, zlshi <zlshi@wh.iov.cn>, Alison Andre <andre@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>, Anna Willoughby <willoughby@ecohealthalliance.org>, Tonie Rocke <trocke@usgs.gov>
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Tel: +65 65168397

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Tuesday, 13 February 2018 1:23 PM
To: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
Subject: Final draft DARPA abstract
Importance: High

Luke, attached is the DARPA abstract.

First thing tomorrow, can you go through the references, and create links to the papers on NCBI. Just turn each reference citation: "(*1*)" for example, into a live link to the paper on NCBI. We only have 2 spare lines, so no room to turn each of these into a PMC numbered ref, as Ralph did for his, but please make sure the citation in parentheses is blue, so it's clear it's a live link on the final pdf.

Ralph, Lina, Peng, Zhengli, Tonie – please give this a quick read to make sure I've not said anything completely wrong. I've had to reduce the text a lot to hit the page limit, but I still think it's a great proposal.

I'll finish off the exec summary slide and <500 wd abstract now.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4473
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

Important: This email is confidential and may be privileged. If you are not the intended recipient, please delete it and notify us immediately; you should not copy or use it for any purpose, nor disclose its contents to any other person. Thank you.

Re: RE: Final draft DARPA abstract, and next steps...

周鹏 <peng.zhou@wh.iov.cn>

Wed 2/21/2018 1:29 AM

To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: Wang Linfa <linfa.wang@duke-nus.edu.sg>; Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

Thanks and fingers crossed!

By the way, there is a tiny mistake on the first line of page 7: "*sinicus* bats at Wuhan Institute of Zoology", should be Wuhan institute of virology. Hope this won't affect too much...

Cheers,
Peng

> -----原始邮件-----
> **发件人:**"Peter Daszak" <daszak@ecohealthalliance.org>
> **发送时间:**2018-02-21 05:47:14 (星期三)
> **收件人:** "Wang Linfa" <linfa.wang@duke-nus.edu.sg>, "Luke Hamel" <hamel@ecohealthalliance.org>, "Jonathon Musser" <musser@ecohealthalliance.org>
> **抄送:** "William B. Karesh" <karesh@ecohealthalliance.org>, "Noam Ross" <ross@ecohealthalliance.org>, "Ralph Baric (rbaric@email.unc.edu)" <rbaric@email.unc.edu>, "周鹏 (peng.zhou@wh.iov.cn)" <peng.zhou@wh.iov.cn>, "Zhengli Shi (zlshi@wh.iov.cn)" <zlshi@wh.iov.cn>, "Alison Andre" <andre@ecohealthalliance.org>, "Aleksei Chmura" <chmura@ecohealthalliance.org>, "Anna Willoughby" <willoughby@ecohealthalliance.org>, "Rocke, Tonie" <trocke@usgs.gov>
> **主题:** RE: Final draft DARPA abstract, and next steps...
>
> Dear All,
>
> Apologies – forgot to send you the final versions of the abstract and the executive summary slide for your records...
>
> Re. the total budget – please don't view that as final – they're planning to give out $40 million over the 3.5 years total to anything from 1 to 6 projects. Given that there were probably 3 or 4 other viable teams in the room at the proposer's conference, I think that $14.8 million is probably

the maximum they would give any project, and it's more likely that they'll fund three or four at 8 million and two or three smaller projects. They'll hopefully give some clearer guidance as we move towards a full proposal.

The due date for proposals is March 27th, 4pm EST (NY time). That means we need to start honing in on our plans for the full proposal. I'm meeting with our team here on Thursday to start working on the next steps, and it would be good to have calls with all of you this week to start refining the ideas and building out the proposal. Luke will follow up with times that work for phone calls.

Thanks again for your openness and clever ideas, and I look forward to working with you all to get the best possible full proposal submitted on time!!!

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4473

www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Peter Daszak
Sent: Tuesday, February 13, 2018 8:15 AM
To: 'Wang Linfa'; Luke Hamel; Jonathon Musser
Cc: William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby; Rocke, Tonie
Subject: RE: Final draft DARPA abstract

Good point Linfa – changed to 'treatment' or 'application' throughout.

Cheers,

Peter

Peter Daszak

President

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pandemics and promote conservation.

From: Wang Linfa [mailto:linfa.wang@duke-nus.edu.sg]
Sent: Tuesday, February 13, 2018 1:10 AM
To: Peter Daszak; Luke Hamel; Jonathon Musser
Cc: William B. Karesh; Noam Ross; Ralph Baric (rbaric@email.unc.edu); 周鹏 (peng.zhou@wh.iov.cn); Zhengli Shi (zlshi@wh.iov.cn); Alison Andre; Aleksei Chmura; Anna Willoughby; Rocke, Tonie
Subject: RE: Final draft DARPA abstract

Hi Peer,

It actually reads well now and I hope the selection committee will "buy in" our brave ideas!

Nothing to add a or change, other than an English question: we used "dampened immunity of bats" in most places, but you use the expression of "damping innate immunity pathways" on page 1. Is there a difference between "damping" and "dampening"?

Thanks

LF

Linfa (Lin-Fa) Wang, PhD FTSE

Professor & Director

Programme in Emerging Infectious Diseases

Duke-NUS Medical School

8 College Road, Singapore 169875

Tel: +65 65168397

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Tuesday, 13 February 2018 1:23 PM
To: Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>;

Wang Linfa <linfa.wang@duke-nus.edu.sg>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
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Peter

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Print Cancel

RE: RE: Final draft DARPA abstract, and next steps...

Peter Daszak <daszak@ecohealthalliance.org>
Wed 2/21/2018 11:01 AM

To: 周鹏 <peng.zhou@wh.iov.cn>
Cc: Wang Linfa <linfa.wang@duke-nus.edu.sg>; Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>

Ouch – great that you spotted that mistake and I'll definitely change that in the full proposal!

Cheers,

Peter

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From: 周鹏 [mailto:peng.zhou@wh.iov.cn]
Sent: Wednesday, February 21, 2018 2:30 AM

PREEMPT call with Peter

Luke Hamel <hamel@ecohealthalliance.org>

Mon 2/26/2018 9:05 AM

To: Rocke, Tonie E <trocke@usgs.gov>

Hi Tonie,

My name is Luke Hamel and I am a Program Assistant at EcoHealth Alliance, helping to coordinate efforts for DARPA PREEMPT. As the full project proposal is due March 27th, Peter was hoping to speak with you in order to discuss further details of the proposal and establish a timeline for moving forward.

While I know this is very short notice, would you be free this **Tue. 2/27 @ 9 AM (OR) 1 PM (ET)** to speak with Peter? If you are not available, could you please let me know when a convenient time would be for you? Thank you very much.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

For our DARPA PREEMPT conversations this week: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status

Peter Daszak <daszak@ecohealthalliance.org>

Tue 2/27/2018 1:13 PM

To: Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; Rocke, Tonie E <trocke@usgs.gov>
Cc: Danielle Anderson (danielle.anderson@duke-nus.edu.sg) <danielle.anderson@duke-nus.edu.sg>; aaron.irving@duke-nus.edu.sg <aaron.irving@duke-nus.edu.sg>; antonette_baric@med.unc.edu <antonette_baric@med.unc.edu>; sims0018@email.unc.edu <sims0018@email.unc.edu>; Luke Hamel <hamel@ecohealthalliance.org>

Dear All,

Good news from DARPA - they like our abstract and we're officially invited for a full proposal. From the attached letter, it looks like they've got a lot of proposals asking for too much $$$, but there are some clear ways we can hedge against any possible cuts. We can talk further about this, and about fleshing out the technical details on our calls this week.

I'm working on scheduling a call with the DARPA team for Thursday of Friday this week - 15 mins to go through how these bullets in the letter above will affect our full proposal. It'll just be me and Luke, but we can think about key questions to ask them..

Re. the full proposal. Luke has taken the abstract text and started populating the full proposal framework (attached), to give us an idea of what we need to write. It's not a huge effort, but it'll have to be technically sound, but still tell the overall 'story' that DARPA want to hear - i.e. we can provide proof-of-concept of blocking spillover based on this novel and interesting approach.

Look forward to talking with all of you.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4474
www.ecohealthalliance.org
@PeterDaszak
@EcoHealthNYC

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-----Original Message-----
From: PREEMPT [mailto:PREEMPT@darpa.mil]
Sen 8:51 AM
To: (b) (6)

Cc: (b) (6)
Subject: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status

(b) (6) :

Thank you for your interest in the Biological Technologies Office's PREventing EMerging Pathogenic Threats (PREEMPT) program. Please find your proposal abstract status attached.

Regards,

BAA Coordinator
Contractor Support to DARPA/BTO
PREEMPT@darpa.mil

(b) (4), (b) (5), (b) (6)

(b) (4), (b) (5), (b) (6)

DARPA – PREEMPT PROPOSAL OUTLINE

NOTE: 36 pages max - 12 pt. font or higher (font can be smaller for tables, charts, figures)

VOLUME I – Technical and Management Proposal

Section I – Administrative

A) Cover Page (labeled "Proposal: Volume I")
B) Official Transmittal Letter

Section II – Detailed Proposal Information

A) Executive Summary (questions/answers below from abstract).

Commented [EA1]: Perhaps some of the more detailed information in this section could be moved to the 'Goals and Impacts' section

1) What is the proposed work attempting to accomplish or do?

We will **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses**. We envisage a scenario whereby the US warfighter is deployed to a security hotspot in SE Asia. As planners choose sites for the mission, they will use an app we will design based on machine-learning models of the ecological and evolutionary potential of bat viruses to spillover. This will allow rapid assessment of the background risk of a site harboring dangerous zoonotic viruses. If there is no alternative site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release **broadscale immune boosting molecules** and chimeric polyvalent spike protein **targeted immune priming treatments** to upregulate the naturally damped innate immune response of bats, and lower viral shedding from bats at the site for a few weeks or months, allowing
our warfighters to execute the operation at lowered risk for spillover.

2) How is it done today, and what are the limitations?

Other than PPE, there is no available current technology to reduce the risk of exposure to novel coronaviruses from bats. Models of bat host capacity to harbor viruses, of ecological and environmental drivers of their emergence, and of the evolutionary potential of different strains to spillover are rudimentary. No vaccines or therapeutics exist for SARSr-CoVs, and exposure mitigation strategies are non-existent. SARSr-CoVs are endemic in Asian, African (*1*), and European bats (*2*) that roost in caves but forage widely at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARSr-CoVs into people in China and have isolated strains capable of producing SARS-like illness in humanized mice that don't respond to antibody treatment or vaccination. These viruses are a clear-and-present danger to our military and to global health security.

3) What is innovative in your approach?

Our group leads the world in predictive models of viral emergence. We will build on our machine-learning models of spillover hotspots, host-pathogen ecological niches and genotype-phenotype mapping by incorporating unique datasets to validate and refine hotspot risk maps of viral emergence in SE Asia and beyond. Our group has shown that bats coexist with lethal viruses by damping innate immunity pathways, likely as an evolutionary adaptation to flight. We will use this insight to design strategies, like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists, to upregulate bat immunity in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover **(broadscale immune boosting strategy)**. We will complement this by treating bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against specific, high-risk viruses **(targeted immune priming strategy)**, especially when their immune response is boosted as above. We will design novel methods to deliver these applications remotely to reduce exposure risk during decontamination.

4) What are the key technical challenges in your approach and how do you plan to overcome these?

Modeling: Previous models have suffered from a lack of data to validate them. We have access to unique datasets that will allow us to validate our approach, including biodiversity surveys of bat caves across S. China, 10+ years of bat viral testing data in China, and 10 other countries (from NIH-NIAID and USAID EPT PREDICT work). Uniquely, we will validate our models of viral evolution/spillover risk using human serology (based on LIPS assays) in local populations that have high (~3%) seroprevalence to bat SARSr-CoVs. *Identifying Immune boosting and priming treatments:* Some of our approaches are novel and challenging (e.g. using CRISPRi to find the negative regulator for bat interferon production), and others are unproven in bats (e.g. Poly IC). We will begin all immune boosting and priming experiments at the beginning of the project, running them simultaneously and competitively, so that we field trial only the most efficient, cost-effective and scalable approaches.

5) Who or what will be affected and what will be the impact?

This will have direct relevance to the warfighter. Potential deployment to regions where SARSr-CoVs exist is high – countries include security hotspots in Asia (e.g. Myanmar, Bangladesh, Pakistan, Korea, Vietnam), Africa and Eastern Europe. The ability to decontaminate and defuse these viruses may prevent potentially devastating illness. These technologies could be adapted to hosts of other bat-origin CoVs (e.g. MERS-CoV, SADS-CoV) and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV), with benefits to livestock production, food security and global public health.

B) **Executive Summary Slide** (must use provided template)

C) **Goals and Impact**

Commented [EA2]: Detailed text from the 'Executive Summary' section may be better suited here

- Clearly describe what the team is trying to achieve and the difference it will make (qualitatively and quantitatively) if successful.
- Describe the innovative aspects of the project in the context of existing capabilities and approaches, clearly delineating the uniqueness and benefits of this project in the context of the state of the art, alternative approaches, and other projects from the past and present.
- Describe how the proposed project is revolutionary and how it significantly rises above the current state of the art.
- Describe the deliverables associated with the proposed project and any plans to commercialize the technology, transition it to a customer, or further the work.

Overview

The SARSr-CoV-bat system, and immune modulation focus: Our group's 15 yrs work on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014 & WIV-1). We have shown they bind and replicate efficiently in primary human lung airway cells and that chimeras with SARSr-CoV spike proteins in a SARS-CoV backbone cause SARS-like illness in humanized mice, with clinical signs that are not reduced by SARS monoclonal therapy or vaccination. We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV (*7,8,9*). We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**. Our work on bat immunology suggests that bats' unique flying ability has led to downregulated innate immune genes, and their ability to coexist with viruses such as SARSr-CoVs, henipa- and filoviruses that are lethal in many other mammals (3). We have identified bat-specific constitutively expressed bat interferon, a dampened STING-interferon production pathway (4, 5), and have identified a series of other innate immunity factors that are dampened in bats (6).

Our bat-CoV system has significant advantages for experimentation and intervention. Firstly, these viruses are fecal-orally transmitted within bat populations, so sampling can be achieved from fresh fecal pellet collection. They are BSL-3, not -4, agents, so that experimental manipulation and infection is simpler. They have frequent spillover events, making it possible to validate predictive models of spillover by sampling people. They are diverse, with frequent recombination and different strains exhibiting differential host cell binding and spillover potential. Finally, we have identified SARSr-CoV strains in a single cave in Yunnan that harbor all of the epidemic SARS-CoV genes. This specific bat population harbors an ideal evolutionary soup that could produce new human strains by high frequency RNA recombination, and thus, it presents a perfect target for next generation, technology-forward intervention strategies.

D) Technical Plan

- Outline and address technical challenges inherent in the approach and possible solutions for overcoming potential problems.
- Provide appropriate measurable milestones (quantitative if possible) and program metrics (see "metrics" attachment) at intermediate stages of the program to demonstrate progress, and a plan for achieving the milestones.
- Demonstrate a deep understanding of the technical challenges.
- Present a credible (even if risky) plan to achieve the program goal.
- Discuss mitigation of technical risk.
- Address TA1 and TA2 proposal content requirements (see "objectives" attachment)

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team, led by Drs. Daszak, Ross, Olival, EHA, will build ecological niche models of environmental and ecological correlates and traits of cave bat communities to predict species composition of bat caves across Southern China, South and SE Asia. We will then use a series of datasets we have built to produce host-virus risk models for the region. These include our unique database of bat host-viral relationships (*7*); biological inventory data on all bat caves in Southern China; and modeled species distribution data for all bats. We will parameterize the model with data from three cave sites in Yunnan, China (one with high-risk SARSr-CoVs, two other control/comparison sites), including: radio- and GPS-telemetry to identify home range and additional roost sites for each bat species; inventory of bat population density, distribution and segregation and their daily, weekly and seasonal changes; viral prevalence and individual viral load; shedding of low- and high-risk SARSr-CoV strains among bat species, age classes, genders; and telemetry and mark-recapture data to assess metapopulation structure and inter-cave connectivity. We will test and validate model predictions of a cave's viral spillover potential with data from prior PREDICT sampling in 7 other Asian countries. At the end of Yr 1, we will produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens in a region. **The 'high-risk bats near me' app** will be updated real-time with surveillance data (e.g. field-deployable iPhone and android compatible echolocation data) from our project and others, to ground-truth and fine-tune its predictive capacity.

The Wuhan Institute of Virology team will test bat fecal, oral, blood and urogenital samples for SARSr-CoVs. We will correlate viral load data from these samples with fresh fecal pellets from individuals and from tarps laid on cave floors. We will rapidly move to fecal pellet assays to reduce roost disturbance. SARSr-CoV spike proteins will be sequenced, analyzed phylogenetically for recombination events, and high-risk viruses (spike proteins close to SARS-CoV) characterized and isolated. The UNC team will reverse-engineer spike proteins to conduct binding assay to human ACE2 (the SARS-CoV receptor). They will culture SARS-like bat coronaviruses to distinguish high-risk strains that can replicate in primary human cells and low risk strains that require exogenous enhancers. Viral spike glycoproteins that bind receptors will be inserted into SARS-CoV backbones, inoculated into human cells and humanized mice to assess capacity to cause SARS-like disease, and to be blocked by monoclonal therapies, the nucleoside analogue inhibitor GS-5734(*8*)or vaccines against SARS-CoV(*8,9,10,11,12,13*).

The EHA modeling team will use these data to **build models of risk of viral evolution and**

spillover. These genotype-to-phenotype machine-learning models will predict viral ability to infect host cells based on genetic traits and results of receptor binding and mouse infection assays. Using data on diversity of spike proteins, recombinant CoVs, and flow of genes within each bat cave via bat movement and migration, we will estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Finally, virus-host relationship and bat home range data will be used to estimate spillover potential-extending models well beyond our field sites. We will then **validate model predictions of viral spillover risk** by **1)** conducting spike protein-based binding and cell culture experiments, and **2)** identifying spillover strains in people near our bat cave sites. Our preliminary work on this shows ~3% seroprevalence to SARSr-CoVs, using a specific ELISA (*14*).We will design LIPS assays to the specific high-and low-zoonotic-risk SARSr-CoVs identified in this project as we have done previously(*15*).We will use banked and newly collected human sera from these populations to test for presence of antibodies to the high-and low-risk SARSr-CoVs identified by our modeling. We will then **model optimal strategies to maximize treatment efficacy** for TA2,using stochastic simulation modeling informed by field and experimental data to characterize viral circulation dynamics in bats. We will estimate frequency and population coverage required for our intervention approaches to suppress viral spillover. We will determine the seasons, locations within a cave, and delivery methods (spray, swab, or automated cave mouth or drone)that will be most effective. Finally we will determine the time period treatment will be effective for, until re-colonization or evolution leads to return of a high-risk SARSr-CoV.

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s)and/or vector(s), to reduce the likelihood of virus transmission into humans.

We will evaluate two approaches to defuse SARS-related CoV spillover potential**: 1) Broadscale Immune Boosting:** using the unique immune damping in bat that our group has discovered, we will apply immune modulators like bat interferon to live bats, to up-regulate their naïve immunity and then assess their ability to suppress viral replication and shedding;**2) Targeted Immune Priming:** building on preliminary development of polyvalent chimeric recombinant SARSr-CoV spike proteins, we will conduct application trials with live bats to assess suppression of replication and shedding of abroad range of dangerous SARS-related CoVs.

Both lines of work will begin in Yr 1and run parallel. Prof. Linfa Wang (Duke-NUS) will lead the immune boosting work, building on his pioneering work on bat immunity (*3*) which shows that the long-term coexistence of bats and their viruses has led to equilibrium between viral replication and host immunity. This is likely due to down-regulation of their innate immune system as a fitness cost of flight (*3*). The weakened functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may allow bats to maintain an effective, but not over-response to viruses (4). A similar finding was observed for bat IFNA, which is less abundant but constitutively expressed without stimulation (5). Given high native SARSr-CoV load in bats, we aim to boost bat innate immunity through the IFN pathway, break the host-virus equilibrium to suppress bat SARSr-CoV replication and shedding.

We will trial the following, concurrently and competitively, for efficiency, cost and scalability: i) Universal bat interferon. Aerosol spraying or intranasal application of IFN or other small molecules reduces viral loads in humans, ferrets and mouse models (16, 17). Interferon has been used clinically when antiviral drugs are unavailable, e.g. against filoviruses (18). Replication of SARSr-CoV is sensitive to interferon treatments, as shown in our previous work

(16); ii) Boosting bat IFN by blocking bat-specific IFN negative regulators. Uniquely, bat IFNA is naturally constitutively expressed but cannot be induced to a high level (5), indicating a negative regulatory factor in the bat interferon production pathway. We will use CRISPRi to identify the negative regulator and then screen for compounds targeting this gene; iii) Activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7-dependent pathways. Our work showing that mutant bat STING restores antiviral functionality suggests these pathways are important in bat-viral coexistence (4). By identifying small molecules to directly activate downstream of STING, we will activate bat interferon and promote viral clearance. A similar strategy will be applied to ssRNA-TLR7-dependent pathways; iv) Activating functional bat IFN production pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been demonstrated in a mouse model for SARS-CoV, IAV and HBV (17, 19); v) Inoculating crude coronavirus fragments to upregulate innate immune responses to specific CoVs – a partial step towards the targeted immune priming work below.

Prof. Ralph Baric (UNC) will lead the immune priming work. He will develop recombinant chimeric spike-proteins (20) from our known SARSr-CoVs, and those we characterize during project DEFUSE. The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain (21). In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles (11, 22,23,24,25). We will test these in controlled lab conditions, taking the best candidate forward for testing in the field. We have built recombinant spike glycoproteins harboring structurally defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broad scale immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, potentially reducing disease risk (and transmission risk to people) in these animals for longer periods (26, 27).

The immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established the only experimental breeding colony of cave bats (Eonycteris spelaea) in SE Asia. This genus is evolutionarily related to Rhinolophus spp. (the hosts of SARSr-CoVs), so we have confidence that results will be transferable. Our initial proof-of-concept tests will be in this experimental colony, extended to a small group of wild-caught Rhinolophus *sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with *Rhinolophus* sp. bats in the BSL-4 facility at CSIRO, AAHL (L.Wang, unpublished results).

Finally, work on a delivery method for our immune boosting and priming molecules will be overseen by Dr. Tonie Rocke at the USGS, National Wildlife Health Center who has previously developed animal vaccines through to licensure (*28*). Using locally acquired insectivorous bats (*29, 30*) we will assess delivery vehicles and methods including: 1) transdermally applied nanoparticles; 2) series of sticky edible gels that bats will groom from themselves and each other; 3) aerosolization via sprayers that could be used in cave settings; 4) automated sprays triggered by timers and movement detectors at critical cave entry points, and 5) sprays delivered by remote controlled drone. We have already used simple gels to vaccinate bats against rabies in the lab (*29*), and hand delivered these containing biomarkers to vampire bats in Peru and Mexico to show they are readily consumed and transferred among bats. In our bat

colony, we will trial delivery vehicles using the biomarker rhodamine B (which marks hair and whiskers upon consumption) to assess uptake. The most optimal approaches will then be tested on wild bats in our three cave sites in Yunnan Province with the most successful immunomodulators from TA2. Fieldwork will be conducted under the auspices of Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance). A small number of bats will be captured and assayed for viral load and immune function after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has had unique access to these sites for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for experimental trials from the Provincial Forestry Department. We expect to be successful, as we have worked with the Forestry Department collaboratively for 10 years, with support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife. EHA has a proven track record of rapidly obtaining IACUC and DoD ACURO approval for bat research.

E) Management Plan

- Provide a summary of expertise of the team, including any subcontractors, and key personnel who will be doing the work. Resumes count against the page count.
- Identify a principal investigator for the project.
- Provide a clear description of the team's organization
- Include an organization chart with the following information, as applicable:
  A) Programmatic relationship of team members
  B) Unique capabilities of team members
  C) Task responsibilities of team members
  D) Teaming strategy among the team members
  E) Key personnel with amount of effort to be expended by each during each year
- Provide a detailed plan for coordination including explicit guidelines for interaction among collaborators/subcontractors of the proposed effort.
- Include risk management approaches.
- Describe any formal teaming agreements that are required to execute this program.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research organization focused on emerging zoonotic diseases. The PI, Dr. Peter Daszak, has 25+ years' experience managing lab, field and modeling research projects on emerging zoonoses. Dr. Daszak will commit 3 months annually to oversee and coordinate all project activities, and lead modeling and analytic work for TA1. Dr. Billy Karesh has 40+ years' experience leading zoonotic and wildlife disease projects, and will commit 1 month annually to manage partnership activities and outreach. Dr. Jon Epstein, with 15 years' experience working

emerging bat zoonoses will coordinate animal trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project. Support staff include field surveillance teams, modeling analysts, and consultants based in Yunnan Province, China, to oversee field trials. The EHA team has worked extensively with all other collaborators: Prof. Wang (15+ years); Dr. Shi (15+ years); Prof. Baric (5+ years) and Dr. Rocke (15+ years). **Subcontracts: #1** to Prof. Ralph Baric, UNC, to oversee reverse engineering of SARSr-CoVs, BSL-3 humanized mouse experimental infections, design and testing of immune priming treatments based on recombinant spike proteins. Assisted by senior personnel Dr. Tim Sheahan, Dr. Amy Sims, and support staff; **#2** to Prof. Linfa Wang, Duke NUS, to oversee the immune boosting approach, captive bat experiments, and analyze immunological and virological responses to immune boosting treatments; **#3** to Dr. Zhengli Shi, Wuhan Institute of Virology, to conduct PCR testing, viral discovery and isolation from bat samples collected in China, spike protein binding assays, and some humanized mouse work, as well as experimental trials on *Rhinolophus* bats. Her team will include Dr. Peng Zhou and support staff; **#4** to Dr. Tonie Rocke, USGS National Wildlife Health Center, to refine delivery mechanisms for both immune boosting and immune priming treatments. With a research technician, Dr. Rocke will use a captive colony of bats at NWHC for initial trials, and oversee cave experiments in China.

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based research organization focused on emerging zoonotic diseases. His >300 scientific papers include the first global map of EID hotspots *(31, 32)*, estimates of unknown viral diversity *(33)*, predictive models of virus-host relationships *(7)*, and evidence of the bat origin of SARS-CoV *(34, 35)* and other emerging viruses *(36,37,38,39)*. He is Chair of the NASEM Forum on Microbial Threats, and is a member of the Executive Committee and the EHA institutional lead for the $130 million USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr. Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Dept. of Epidemiology and Dept. of Microbiology & Immunology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, cross species transmission and pathogenesis. His group has developed a platform strategy to access the potential "pre-epidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease *(8,9,10,11,12,13)*.

F) Capabilities

- Describe organizational experience in relevant subject area(s), existing intellectual property, specialized facilities, and any Government-furnished materials or information.

- Discuss any work in closely related research areas and previous accomplishments.

(The following information was taken from the 'Goals and Impact' section of the document).

The SARSr-CoV-bat system, and immune modulation focus: Our group's 15 yrs work on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014 & WIV-1). We have shown they bind and replicate efficiently in primary human lung airway cells and that chimeras with SARSr-CoV spike proteins in a SARS-CoV backbone cause SARS-like illness in humanized mice, with clinical signs that are not reduced by SARS monoclonal therapy or vaccination. We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV *(7,8,9)*. We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**. Our work on bat immunology suggests that bats' unique flying ability has led to downregulated innate immune genes, and their ability to coexist with viruses such as SARSr-CoVs, henipa- and filoviruses that are lethal in many other mammals (3). We have identified bat-specific constitutively expressed bat interferon, a dampened STING-interferon production pathway (4, 5), and have identified a series of other innate immunity factors that are dampened in bats (6).

G) Statement of Work (SOW)

- Provide a detailed task breakdown, citing specific tasks and their connection to the interim milestones and program metrics.

- Each phase of the program (Phase I base and Phase II option) should be separately defined in the SOW and each task should be identified by TA (1 or 2).

NOTE: The SOW must not include proprietary information.

- For each task/subtask, provide:
    - A detailed description of the approach to be taken to accomplish each defined task/subtask.
    - Identification of the primary organization responsible for task execution (prime contractor, subcontractor(s), consultant(s), by name).
    - A measurable milestone, i.e., a deliverable, demonstration, or other event/activity that marks task completion. Include quantitative metrics.
    - A definition of all deliverables (e.g., data, reports, software) to be provided to the Government in support of the proposed tasks/subtasks.

Phase I:

***(TA1)* Task 1: Collect ecological and environmental data from Yunnan Province cave sites.**

Description and execution:

Commented [EA3]: I've taken tasks from the 'Technical Plan' section above, but these tasks may need to be reorganized according to Phase (I or II).

Also, feel free to alter names of tasks, as well as task/subtask classifications

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

***(TA1) Task* 2: Construct niche models to predict species composition of bat caves across South and Southeast Asia**

Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

***(TA1)* Subtask 2.1: Construct host-pathogen risk models**

Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

***(TA1)* Subtask 2.2: Develop prototype app for the warfighter**

Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

(TA1) **Task 3: Analyze bat samples for SARSr-CoVs**

Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

(TA1) **Subtask 3.1 Develop recombinant chimeric spike proteins from characterized SARSr-CoVs**

Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

(TA1) **Subtask 3.2 Conduct assays in humanized mice to identify high-risk SARSr-CoV strains**

Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

(TA2) **Task 4: Trial experimental approaches aimed towards 'Broadscale Immune Boosting' using experimental bat colonies**

Description and execution:

Preliminary Data:

Organization leading task:

Commented [EA4]: This task may carry over into Phase II. Might want to break up between Phases I and II.

Progress Metrics:

Deliverable(s):

***(TA2)* Task 5: Trial experimental approaches aimed towards 'Immune Targeting' using experimental bat colonies**

Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

Commented [EA5]: This task may carry over into Phase II. Might want to break up between Phases I and II.

***(TA2)* Task 6: Develop and assess delivery methods for immune boosting and priming molecules**

Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

***(TA1)* Task 7: Construct genotype-to-phenotype machine learning models to predict risk of viral evolution and spillover**

Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

(TA2) Subtask 7.1: Validate model predictions of viral spillover risk

Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

Phase II:

(TA1) **Task 1: Design LIPS assays to specific high- and low- zoonotic risk SARSr-CoVs**
Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

(TA1) **Task 2: Construct models to maximize efficacy of intervention approaches**
Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

(TA2) **Task 3: Deploy most effective molecule delivery methods on bat colonies of Yunnan Province caves**
Description and execution:

Preliminary Data:

Organization leading task:

Progress Metrics:

Deliverable(s):

H) Schedule and Milestones

- Provide a detailed schedule showing tasks (task name, duration, work breakdown structure element as applicable, performing organization), milestones, and the interrelationships among tasks.

  NOTE: Task structure must be consistent with that in the SOW.

- Measurable milestones should be clearly articulated and defined in time relative to the start of the project.

I) PREEMPT Transition Plan

- Indicate the types of partners (e.g., government, private industry, non-profit)

- Submit a timeline with incremental milestones toward successful engagement.
  NOTE: begin transition activities during the early stages of the program (Phase I).

- Describe any potential DARPA roles.

J) PREEMPT Risk Mitigation Plan

- Provide the following:

  - An assessment of potential risks to public health, agriculture, plants, animals, the environment, and national security.

  - Guidelines the proposer will follow to ensure maximal biosafety and biosecurity.

  - A communication plan that addresses content, timing, and the extent of distribution of potentially sensitive dual-use information. The plan must also address how input from DARPA, other government, and community stakeholders will be taken into account in decisions regarding communication and publication of potentially sensitive dual-use information.

K) Ethical, Legal, Societal Implications (ELSI)

- Address potential ethical, legal, and societal implications of the proposed technology.

Section III – Additional Information (doesn't count against 36 pg. limit)

Commented [EA6]: Description from the BAA:

PREEMPT Transition Plan
Proposers must include a PREEMPT Technology Transition Plan. Proposers must indicate the types of partners (e.g., government, private industry, non-profit) they plan to pursue and submit a timeline with incremental milestones toward successful engagement. Proposers should begin transition activities during the early stages of the program (Phase I). Awardees must include
DARPA in the development of transition relationships. If the transition plan includes a start-up company, a business development strategy must be included as well. The extent by which the proposed intellectual property (IP) rights will impede the Government's ability to transition thetechnology will be considered in the proposal evaluation.

A) Brief Bibliography (no page limit indicated – can be published/unpublished)
B) Up to 3 relevant papers attached (optional)

Re: Email to Tonie Rocke (PREEMPT)

Brian Baker <baker@ecohealth.net>

Wed 2/28/2018 8:35 AM

To: Rocke, Tonie E <trocke@usgs.gov>

Sure! That works great.

I believe you'll be using EHA's Domestic Line to call in, number and passcode below for your convenience:

1 (719) 785-9461
97848#

I'll let you know if anything changes.

Thanks again,

Brian

> On Feb 27, 2018, at 10:28 PM, Rocke, Tonie <trocke@usgs.gov> wrote:
>
> Could we do it at 3 PM my time? I have another meeting early afternoon. Thanks -Tonie
>
> On Tue, Feb 27, 2018 at 3:25 PM, Brian Baker <baker@ecohealth.net> wrote:
>> Yes, Friday works great. How about 3pm EST (2pm your time)?
>>
>> —
>> Brian Baker
>> Assistant Managing Editor, *EcoHealth*
>> 460 West 34th Street, 17th Floor
>> New York, NY 10001
>> 1.212.380.4498 (direct)
>> brian.hartman.baker (Skype)
>>
>> Website: www.ecohealth.net
>> Submissions and Log-in: https://mc.manuscriptcentral.com/ecohealth
>> Author Instructions: http://www.ecohealth.net/submit.php
>>
>>> On Feb 27, 2018, at 3:46 PM, Rocke, Tonie <trocke@usgs.gov> wrote:
>>>
>>> HI Brian: I'm out until Thursday evening. Friday afternoon would be OK if that works for you. -Tonie
>>>
>>> On Tue, Feb 27, 2018 at 2:39 PM, Brian Baker <baker@ecohealth.net> wrote:
>>>> Hi Tonie,
>>>>
>>>> Sorry for the double email!

My coworker Luke (cc'd here) asked if I could pass along his note to you regarding scheduling a call with Peter for later this week. Apologies if we're being pushy.

Safe travels and thanks,

Brian

—

Brian Baker
Assistant Managing Editor, *EcoHealth*
460 West 34th Street, 17th Floor
New York, NY 10001
1.212.380.4498 (direct)
brian.hartman.baker (Skype)

Website: www.ecohealth.net
Submissions and Log-in: https://mc.manuscriptcentral.com/ecohealth
Author Instructions: http://www.ecohealth.net/submit.php

Begin forwarded message:

From: Luke Hamel <hamel@ecohealthalliance.org>
Subject: Email to Tonie Rocke (PREEMPT)
Date: February 27, 2018 at 3:25:22 PM EST
To: Brian Baker <baker@ecohealthalliance.org>

Subject Title: PREEMPT call with Peter

Hi Tonie,

My name is Luke Hamel and I am a Program Assistant at EcoHealth Alliance, helping to coordinate efforts for DARPA PREEMPT.

As the full project proposal is due March 27th, **Peter was hoping to speak with you in order to discuss further details of the proposal and establish a timeline for moving forward.**

If you are available, Peter would like to speak with you on either of the following dates:

Thu. 3/1 between 11:30 AM - 3:00 PM (ET)
Fri. 3/2 between 1:00 PM - 5:00 PM (ET)

Could you please respond with a time that works for you, or provide an alternative date/time that is more convenient? Thank you very much.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)

(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

RE: For our DARPA PREEMPT conversations this week: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status

Peter Daszak <daszak@ecohealthalliance.org>

Thu 3/1/2018 12:00 AM

To: Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; Rocke, Tonie E <trocke@usgs.gov>
Cc: Danielle Anderson (danielle.anderson@duke-nus.edu.sg) <danielle.anderson@duke-nus.edu.sg>; aaron.irving@duke-nus.edu.sg <aaron.irving@duke-nus.edu.sg>; Baric, Toni C <antoinette_baric@med.unc.edu>; sims0018@email.unc.edu <sims0018@email.unc.edu>; Luke Hamel <hamel@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>

Dear All,

Please see attached, a revised template for the PREEMPT Full Proposal. For the moment, please focus your attention on Section G (Statement of Work), as this is where technical information for all tasks and subtasks must be detailed. The other sections can remain as they are for now – I'll edit these.

Please start drafting your sections as indicated to expand the details of the prelim data, work plans etc. to fill out the tasks/subtasks for which your institution has been highlighted. Duke-NUS and Wuhan (Peng and Zhengli) – I'm assuming you're going to draft things together as possible, so I've put the names of both together. If this isn't correct – please arrange among yourselves to decide who will lead which part.

Regarding the writing of each section, keep in mind the following:

1. If you want to add a subtask, or change the titles, please feel free, but make a note (comment box) so we know that you changed it – no need to use 'track changes'
2. Include as much detailed, technical information as necessary. Don't worry about the page limit. I can remove text as needed, later on.
3. For each task/subtask that you write, be sure to include preliminary data if applicable (e.g. no. of caves sampled, no. of samples collected, etc.)
4. Include any relevant figures, tables or charts – the more the better, we can always delete/edit/shrink down later on
5. We think the 'Description and execution' bullet is DARPA-speak for 'Research Plan' or 'Plan of work', i.e. where you lay out the strategy, the rationale, and the technical details of how you are going to achieve each goal.
6. Please put in some ideas for the bullets on 'Progress metrics' and 'deliverables'. We'll make sure we go back to these after the first drafts are collated, so that we write this in a uniform way that will appeal to DARPA.
7. Be sure to include any relevant references. Please use EndNote if possible; otherwise send list of references to me (Peter), cc'ing Luke Hamel. Note that some of the references are embedded as links to Pubmed webpages. I'll be converting these back to Endnote later on, so ignore for now. We can insert as many references as we like because they're not included in the page length.
8. Please send drafts back to me by Wed. 3rd March (Eastern time – NYC time). Earlier if possible! As soon as they start coming in, I'll be incorporating text, editing and adding to different sections so we have a good draft by the end of that week.

Additionally, **please send me a list of all personnel you plan to include on your team, as soon as you are able**. Along with names, please provide (1) Number of months to be committed to project, and (2) % effort for each member of your team (including yourself). This can be approximate right now and don't worry about this affecting budget – it won't - we're going to keep that level as suggested previously…

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4474
www.ecohealthalliance.org
@PeterDaszak
@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

-----Original Message-----
From: Peter Daszak
Sent: Tuesday, February 27, 2018 2:14 PM
To: Zhengli Shi (zlshi@wh.iov.cn); Ralph Baric (rbaric@email.unc.edu); 周鹏 (peng.zhou@wh.iov.cn); 'Wang Linfa'; Rocke, Tonie
Cc: Danielle Anderson (danielle.anderson@duke-nus.edu.sg); 'aaron.irving@duke-nus.edu.sg'; 'antonette_baric@med.unc.edu'; 'sims0018@email.unc.edu'; Luke Hamel (hamel@ecohealthalliance.org)
Subject: For our DARPA PREEMPT conversations this week: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status
Importance: High

Dear All,

Good news from DARPA - they like our abstract and we're officially invited for a full proposal. From the attached letter, it looks like they've got a lot of proposals asking for too much $$$, but there are some clear ways we can hedge against any possible cuts. We can talk further about this, and about fleshing out the technical details on our calls this week.

I'm working on scheduling a call with the DARPA team for Thursday of Friday this week - 15 mins to go through how these bullets in the letter above will affect our full proposal. It'll just be me and Luke, but we can think about key questions to ask them..

Re. the full proposal. Luke has taken the abstract text and started populating the full proposal framework (attached), to give us an idea of what we need to write. It's not a huge effort, but it'll have to be technically sound, but still tell the overall 'story' that DARPA want to hear - i.e. we can provide proof-of-concept of blocking spillover based on this novel and interesting approach.

Look forward to talking with all of you.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4474
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@PeterDaszak
@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

-----Original Message-----
From: PREEMPT [mailto:PREEMPT@darpa.mil]
Sent: Tuesday, February 27, 2018 8:51 AM
To: (b) (6)
Cc: (b) (6)
Subject: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status

(b) (6)

Thank you for your interest in the Biological Technologies Office's PREventing EMerging Pathogenic Threats (PREEMPT) program. Please find your proposal abstract status attached.

Regards,

BAA Coordinator
Contractor Support to DARPA/BTO
PREEMPT@darpa.mil

DARPA – PREEMPT PROPOSAL OUTLINE

NOTE: 36 pages max - 12 pt. font or higher (font can be smaller for tables, charts, figures)

VOLUME I – Technical and Management Proposal

Section I – Administrative

A) Cover Page (labeled "Proposal: Volume I")
B) Official Transmittal Letter

Section II – Detailed Proposal Information

A) Executive Summary (questions/answers below from abstract).

Commented [EA1]: Perhaps some of the more detailed information in this section could be moved to the 'Goals and Impacts' section

1. What is the proposed work attempting to accomplish or do?
We will **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses**. We envisage a scenario whereby the US warfighter is deployed to a security hotspot in SE Asia. As planners choose sites for the mission, they will use an app we will design based on machine-learning models of the ecological and evolutionary potential of bat viruses to spillover. This will allow rapid assessment of the background risk of a site harboring dangerous zoonotic viruses. If there is no alternative site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release **broadscale immune boosting molecules** and chimeric polyvalent spike protein **targeted immune priming treatments** to upregulate the naturally damped innate immune response of bats, and lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?
Other than PPE, there is no available current technology to reduce the risk of exposure to novel coronaviruses from bats. Models of bat host capacity to harbor viruses, of ecological and environmental drivers of their emergence, and of the evolutionary potential of different strains to spillover are rudimentary. No vaccines or therapeutics exist for SARSr-CoVs, and exposure mitigation strategies are non-existent. SARSr-CoVs are endemic in Asian, African (*1*), and European bats (*2*) that roost in caves but forage widely at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARSr-CoVs into people in China and have isolated strains capable of producing SARS-like illness in humanized mice that don't respond to antibody treatment or vaccination. These viruses are a clear-and-present danger to our military and to global health security.

3) What is innovative in your approach?
Our group leads the world in predictive models of viral emergence. We will build on our machine-learning models of spillover hotspots, host-pathogen ecological niches and genotype-phenotype mapping by incorporating unique datasets to validate and refine hotspot risk maps of viral emergence in SE Asia and beyond. Our group has shown that bats coexist with lethal viruses by damping innate immunity pathways, likely as an evolutionary adaptation to flight. We will use this insight to design strategies, like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists, to upregulate bat immunity in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover **(broadscale immune boosting strategy)**. We will complement this by treating bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against specific, high-risk viruses **(targeted immune priming strategy)**, especially when their immune response is boosted as above. We will design novel methods to deliver these applications remotely to reduce exposure risk during decontamination.

4) What are the key technical challenges in your approach and how do you plan to overcome these?
Modeling: Previous models have suffered from a lack of data to validate them. We have access to unique datasets that will allow us to validate our approach, including biodiversity surveys of bat caves across S. China, 10+ years of bat viral testing data in China, and 10 other countries (from NIH-NIAID and USAID EPT PREDICT work). Uniquely, we will validate our models of viral evolution/spillover risk using human serology (based on LIPS assays) in local populations that have high (~3%) seroprevalence to bat SARSr-CoVs. *Identifying Immune boosting and priming treatments:* Some of our approaches are novel and challenging (e.g. using CRISPRi to find the negative regulator for bat interferon production), and others are unproven in bats (e.g. Poly IC). We will begin all immune boosting and priming experiments at the beginning of the project, running them simultaneously and competitively, so that we field trial only the most efficient, cost-effective and scalable approaches.

5) Who or what will be affected and what will be the impact?
This will have direct relevance to the warfighter. Potential deployment to regions where SARSr-CoVs exist is high – countries include security hotspots in Asia (e.g. Myanmar, Bangladesh, Pakistan, Korea, Vietnam), Africa and Eastern Europe. The ability to decontaminate and defuse these viruses may prevent potentially devastating illness. These technologies could be adapted to hosts of other bat-origin CoVs (e.g. MERS-CoV, SADS-CoV) and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV), with benefits to livestock production, food security and global public health.

B) **Executive Summary Slide** (must use provided template)

C) **Goals and Impact**

Commented [EA2]: Detailed text from the 'Executive Summary' section may be better suited here

- Clearly describe what the team is trying to achieve and the difference it will make (qualitatively and quantitatively) if successful.

- Describe the innovative aspects of the project in the context of existing capabilities and approaches, clearly delineating the uniqueness and benefits of this project in the context of the state of the art, alternative approaches, and other projects from the past and present.

- Describe how the proposed project is revolutionary and how it significantly rises above the current state of the art.

- Describe the deliverables associated with the proposed project and any plans to commercialize the technology, transition it to a customer, or further the work.

Overview

The SARSr-CoV-bat system, and immune modulation focus: Our group's 15 yrs work on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014 & WIV-1). We have shown they bind and replicate efficiently in primary human lung airway cells and that chimeras with SARSr-CoV spike proteins in a SARS-CoV backbone cause SARS-like illness in humanized mice, with clinical signs that are not reduced by SARS monoclonal therapy or vaccination. We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV (*7,8,9*). We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**. Our work on bat immunology suggests that bats' unique flying ability has led to downregulated innate immune genes, and their ability to coexist with viruses such as SARSr-CoVs, henipa- and filoviruses that are lethal in many other mammals (*3*). We have identified bat-specific constitutively expressed bat interferon, a dampened STING-interferon production pathway (*4, 5*), and have identified a series of other innate immunity factors that are dampened in bats (*6*).

Our bat-CoV system has significant advantages for experimentation and intervention. Firstly, these viruses are fecal-orally transmitted within bat populations, so sampling can be achieved from fresh fecal pellet collection. They are BSL-3, not -4, agents, so that experimental manipulation and infection is simpler. They have frequent spillover events, making it possible to validate predictive models of spillover by sampling people. They are diverse, with frequent recombination and different strains exhibiting differential host cell binding and spillover potential. Finally, we have identified SARSr-CoV strains in a single cave in Yunnan that harbor all of the epidemic SARS-CoV genes. This specific bat population harbors an ideal evolutionary soup that could produce new human strains by high frequency RNA recombination, and thus, it presents a perfect target for next generation, technology-forward intervention strategies.

D) Technical Plan

- Outline and address technical challenges inherent in the approach and possible solutions for overcoming potential problems.

- Provide appropriate measurable milestones (quantitative if possible) and program metrics (see "metrics" attachment) at intermediate stages of the program to demonstrate progress, and a plan for achieving the milestones.
- Demonstrate a deep understanding of the technical challenges.
- Present a credible (even if risky) plan to achieve the program goal.
- Discuss mitigation of technical risk.
- Address TA1 and TA2 proposal content requirements (see "objectives" attachment)

TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region

The DEFUSE modeling and analytics team, led by Drs. Daszak, Ross, Olival, EHA, will build ecological niche models of environmental and ecological correlates and traits of cave bat communities to predict species composition of bat caves across Southern China, South and SE Asia. We will then use a series of datasets we have built to produce host-virus risk models for the region. These include our unique database of bat host-viral relationships (*7*); biological inventory data on all bat caves in Southern China; and modeled species distribution data for all bats. We will parameterize the model with data from three cave sites in Yunnan, China (one with high-risk SARSr-CoVs, two other control/comparison sites), including: radio- and GPS-telemetry to identify home range and additional roost sites for each bat species; inventory of bat population density, distribution and segregation and their daily, weekly and seasonal changes; viral prevalence and individual viral load; shedding of low- and high-risk SARSr-CoV strains among bat species, age classes, genders; and telemetry and mark-recapture data to assess metapopulation structure and inter-cave connectivity. We will test and validate model predictions of a cave's viral spillover potential with data from prior PREDICT sampling in 7 other Asian countries. At the end of Yr 1, we will produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens in a region. **The 'high-risk bats near me' app** will be updated real-time with surveillance data (e.g. field-deployable iPhone and android compatible echolocation data) from our project and others, to ground-truth and fine-tune its predictive capacity.

Commented [EA3]: SDM with additional set of variables (Carlos thinks this will work)

The Wuhan Institute of Virology team will test bat fecal, oral, blood and urogenital samples for SARSr-CoVs. We will correlate viral load data from these samples with fresh fecal pellets from individuals and from tarps laid on cave floors. We will rapidly move to fecal pellet assays to reduce roost disturbance. SARSr-CoV spike proteins will be sequenced, analyzed phylogenetically for recombination events, and high-risk viruses (spike proteins close to SARS-CoV) characterized and isolated. The UNC team will reverse-engineer spike proteins to conduct binding assay to human ACE2 (the SARS-CoV receptor). They will culture SARS-like bat coronaviruses to distinguish high-risk strains that can replicate in primary human cells and low risk strains that require exogenous enhancers. Viral spike glycoproteins that bind receptors will be inserted into SARS-CoV backbones, inoculated into human cells and humanized mice to assess capacity to cause SARS-like disease, and to be blocked by monoclonal therapies, the nucleoside analogue inhibitor GS-5734 (*8*) or vaccines against SARS-CoV (*8,9,10,11,12,13*).

The EHA modeling team will use these data to **build models of risk of viral evolution and spillover**. These genotype-to-phenotype machine-learning models will predict viral ability

to infect host cells based on genetic traits and results of receptor binding and mouse infection assays. Using data on diversity of spike proteins, recombinant CoVs, and flow of genes within each bat cave via bat movement and migration, we will estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Finally, virus-host relationship and bat home range data will be used to estimate spillover potential - extending models well beyond our field sites. We will then **validate model predictions of viral spillover risk** by **1)** conducting spike protein-based binding and cell culture experiments, and **2)** identifying spillover strains in people near our bat cave sites. Our preliminary work on this shows ~3% seroprevalence to SARSr-CoVs, using a specific ELISA *(14).* We will design LIPS assays to the specific high- and low- zoonotic-risk SARSr-CoVs identified in this project as we have done previously *(15).* We will use banked and newly collected human sera from these populations to test for presence of antibodies to the high- and low-risk SARSr-CoVs identified by our modeling. We will then **model optimal strategies to maximize treatment efficacy** for TA2, using stochastic simulation modeling informed by field and experimental data to characterize viral circulation dynamics in bats. We will estimate frequency and population coverage required for our intervention approaches to suppress viral spillover. We will determine the seasons, locations within a cave, and delivery methods (spray, swab, or automated cave mouth or drone) that will be most effective. Finally we will determine the time period treatment will be effective for, until re-colonization or evolution leads to return of a high-risk SARSr-CoV.

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s)and/or vector(s), to reduce the likelihood of virus transmission into humans. We will evaluate two approaches to defuse SARS-related CoV spillover potential**: 1) Broadscale Immune Boosting:** using the unique immune damping in bats that our group has discovered, we will apply immune modulators like bat interferon to live bats, to up-regulate their naïve immunity and then assess their ability to suppress viral replication and shedding; **2) Targeted Immune Priming:** building on preliminary development of polyvalent chimeric recombinant SARSr-CoV spike proteins, we will conduct application trials with live bats to assess suppression of replication and shedding of a broad range of dangerous SARS-related CoVs.

Both lines of work will begin in Yr 1 and run parallel. Prof. Linfa Wang (Duke-NUS) will lead the immune boosting work, building on his pioneering work on bat immunity *(3)* which shows that the long-term coexistence of bats and their viruses has led to equilibrium between viral replication and host immunity. This is likely due to down-regulation of their innate immune system as a fitness cost of flight *(3)*. The weakened functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may allow bats to maintain an effective, but not over-response to viruses *(4)*. A similar finding was observed for bat IFNA, which is less abundant but constitutively expressed without stimulation *(5)*. Given high native SARSr-CoV load in bats, we aim to boost bat innate immunity through the IFN pathway, break the host-virus equilibrium to suppress bat SARSr-CoV replication and shedding.

We will trial the following, concurrently and competitively, for efficiency, cost and scalability: **i)** Universal bat interferon. Aerosol spraying or intranasal application of IFN or other small molecules reduces viral loads in humans, ferrets and mouse models *(16, 17).* Interferon has been used clinically when antiviral drugs are unavailable, e.g. against filoviruses *(18).* Replication of SARSr-CoV is sensitive to interferon treatments, as shown in our previous work

(16); **ii)** Boosting bat IFN by blocking bat-specific IFN negative regulators. Uniquely, bat IFNA is naturally constitutively expressed but cannot be induced to a high level *(5)*, indicating a negative regulatory factor in the bat interferon production pathway. We will use CRISPRi to identify the negative regulator and then screen for compounds targeting this gene; **iii)** Activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7-dependent pathways. Our work showing that mutant bat STING restores antiviral functionality suggests these pathways are important in bat-viral coexistence *(4)*. By identifying small molecules to directly activate downstream of STING, we will activate bat interferon and promote viral clearance. A similar strategy will be applied to ssRNA-TLR7-dependent pathways; **iv)** Activating functional bat IFN production pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been demonstrated in a mouse model for SARS-CoV, IAV and HBV *(17, 19)*; **v)** Inoculating crude coronavirus fragments to upregulate innate immune responses to specific CoVs – a partial step towards the targeted immune priming work below.

Prof. Ralph Baric (UNC) will lead the immune priming work. He will develop recombinant chimeric spike-proteins *(20)* from our known SARSr-CoVs, and those we characterize during project DEFUSE. The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain *(21)*. In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles *(11, 22,23,24,25)*. We will test these in controlled lab conditions, taking the best candidate forward for testing in the field. We have built recombinant spike glycoproteins harboring structurally defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broad scale immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, potentially reducing disease risk (and transmission risk to people) in these animals for longer periods *(26, 27)*.

The immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established the only experimental breeding colony of cave bats (*Eonycteris spelaea*) in SE Asia. This genus is evolutionarily related to *Rhinolophus* spp. (the hosts of SARSr-CoVs), so we have confidence that results will be transferable. Our initial proof-of-concept tests will be in this experimental colony, extended to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with *Rhinolophus* sp. bats in the BSL-4 facility at CSIRO, AAHL (L.Wang, unpublished results).

Finally, work on a delivery method for our immune boosting and priming molecules will be overseen by Dr. Tonie Rocke at the USGS, National Wildlife Health Center who has previously developed animal vaccines through to licensure *(28)*. Using locally acquired insectivorous bats *(29, 30)* we will assess delivery vehicles and methods including: 1) transdermally applied nanoparticles; 2) series of sticky edible gels that bats will groom from themselves and each other; 3) aerosolization via sprayers that could be used in cave settings; 4) automated sprays triggered by timers and movement detectors at critical cave entry points,

and 5) sprays delivered by remote controlled drone. We have already used simple gels to vaccinate bats against rabies in the lab *(29)*, and hand delivered these containing biomarkers to vampire bats in Peru and Mexico to show they are readily consumed and transferred among bats. In our bat colony, we will trial delivery vehicles using the biomarker rhodamine B (which marks hair and whiskers upon consumption) to assess uptake. The most optimal approaches will then be tested on wild bats in our three cave sites in Yunnan Province with the most successful immunomodulators from TA2. Fieldwork will be conducted under the auspices of Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance). A small number of bats will be captured and assayed for viral load and immune function after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has had unique access to these sites for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for experimental trials from the Provincial Forestry Department. We expect to be successful, as we have worked with the Forestry Department collaboratively for 10 years, with support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife. EHA has a proven track record of rapidly obtaining IACUC and DoD ACURO approval for bat research.

E) Management Plan

- Provide a summary of expertise of the team, including any subcontractors, and key personnel who will be doing the work. Resumes count against the page count.
- Identify a principal investigator for the project.
- Provide a clear description of the team's organization
- Include an organization chart with the following information, as applicable:
  A) Programmatic relationship of team members
  B) Unique capabilities of team members
  C) Task responsibilities of team members
  D) Teaming strategy among the team members
  E) Key personnel with amount of effort to be expended by each during each year
- Provide a detailed plan for coordination including explicit guidelines for interaction among collaborators/subcontractors of the proposed effort.
- Include risk management approaches.
- Describe any formal teaming agreements that are required to execute this program.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research organization focused on emerging zoonotic diseases. The PI, Dr. Peter Daszak, has 25+ years' experience managing lab, field and modeling research projects on emerging zoonoses.

Dr. Daszak will commit 3 months annually to oversee and coordinate all project activities, and lead modeling and analytic work for TA1. Dr. Billy Karesh has 40+ years' experience leading zoonotic and wildlife disease projects, and will commit 1 month annually to manage partnership activities and outreach. Dr. Jon Epstein, with 15 years' experience working emerging bat zoonoses will coordinate animal trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project. Support staff include field surveillance teams, modeling analysts, and consultants based in Yunnan Province, China, to oversee field trials. The EHA team has worked extensively with all other collaborators: Prof. Wang (15+ years); Dr. Shi (15+ years); Prof. Baric (5+ years) and Dr. Rocke (15+ years). **Subcontracts: #1** to Prof. Ralph Baric, UNC, to oversee reverse engineering of SARSr-CoVs, BSL-3 humanized mouse experimental infections, design and testing of immune priming treatments based on recombinant spike proteins. Assisted by senior personnel Dr. Tim Sheahan, Dr. Amy Sims, and support staff; **#2** to Prof. Linfa Wang, Duke NUS, to oversee the immune boosting approach, captive bat experiments, and analyze immunological and virological responses to immune boosting treatments; **#3** to Dr. Zhengli Shi, Wuhan Institute of Virology, to conduct PCR testing, viral discovery and isolation from bat samples collected in China, spike protein binding assays, and some humanized mouse work, as well as experimental trials on *Rhinolophus* bats. Her team will include Dr. Peng Zhou and support staff; **#4** to Dr. Tonie Rocke, USGS National Wildlife Health Center, to refine delivery mechanisms for both immune boosting and immune priming treatments. With a research technician, Dr. Rocke will use a captive colony of bats at NWHC for initial trials, and oversee cave experiments in China.

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based research organization focused on emerging zoonotic diseases. His >300 scientific papers include the first global map of EID hotspots *(31, 32)*, estimates of unknown viral diversity *(33)*, predictive models of virus-host relationships *(7)*, and evidence of the bat origin of SARS-CoV *(34, 35)* and other emerging viruses *(36,37,38,39)*. He is Chair of the NASEM Forum on Microbial Threats, and is a member of the Executive Committee and the EHA institutional lead for the $130 million USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr. Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Dept. of Epidemiology and Dept. of Microbiology & Immunology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, cross species transmission and pathogenesis. His group has developed a platform strategy to access the potential "pre-epidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease *(8,9,10,11,12,13)*.

Prof. Linfa Wang is a

Prof. Zhengli Shi has over xx years experience….

Dr. Tonie Rocke is a

Dr. Peng Zhou is a

Dr. Danielle Anderson is a

Please follow the same format and create Bios for all other personnel with Ph.D and higher. Peter Daszak will then work out how much space we have and decide who to include…

F) Capabilities

- Describe organizational experience in relevant subject area(s), existing intellectual property, specialized facilities, and any Government-furnished materials or information.

- Discuss any work in closely related research areas and previous accomplishments.

(The following information was taken from the 'Goals and Impact' section of the abstract we submitted).

The SARSr-CoV-bat system, and immune modulation focus: Our group's 15 yrs work on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014 & WIV-1). We have shown they bind and replicate efficiently in primary human lung airway cells and that chimeras with SARSr-CoV spike proteins in a SARS-CoV backbone cause SARS-like illness in humanized mice, with clinical signs that are not reduced by SARS monoclonal therapy or vaccination. We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV (*7,8,9*). We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**. Our work on bat immunology suggests that bats' unique flying ability has led to downregulated innate immune genes, and their ability to coexist with viruses such as SARSr-CoVs, henipa- and filoviruses that are lethal in many other mammals (*3*). We have identified bat-specific constitutively expressed bat interferon, a dampened STING-interferon production pathway (*4, 5*), and have identified a series of other innate immunity factors that are dampened in bats (*6*).

G) Statement of Work (SOW)

- Provide a detailed task breakdown, citing specific tasks and their connection to the interim milestones and program metrics.

- Each phase of the program (Phase I base and Phase II option) should be separately defined in the SOW and each task should be identified by TA (1 or 2).

NOTE: The SOW must not include proprietary information.

- For each task/subtask, provide:
    - A detailed description of the approach to be taken to accomplish each defined task/subtask.
    - Identification of the primary organization responsible for task execution (prime contractor, subcontractor(s), consultant(s), by name).
    - A measurable milestone, i.e., a deliverable, demonstration, or other event/activity that marks task completion. Include quantitative metrics.
    - A definition of all deliverables (e.g., data, reports, software) to be provided to the Government in support of the proposed tasks/subtasks.

Phase I:

(TA1) **Task 1: Collect ecological and environmental data from Yunnan Province cave sites.**

Description and execution:

Preliminary Data:

Organization leading task: EcoHealth Alliance

Progress Metrics:

Deliverable(s):

(TA1) Task **2: Construct niche models to predict species composition of bat caves across South and Southeast Asia**

Description and execution:

Preliminary Data:

Organization leading task: EcoHealth Alliance

Progress Metrics:

Deliverable(s):

***(TA1)* Subtask 2.1: Construct, test and validate host-pathogen risk models**

Description and execution:

Preliminary Data:

Organization leading task: EcoHealth Alliance

Progress Metrics:

Deliverable(s):

***(TA1)* Subtask 2.2: Develop prototype app for the warfighter**

Description and execution:

Preliminary Data:

Organization leading task: EcoHealth Alliance

Progress Metrics:

Deliverable(s):

***(TA1)* Task 3: Analyze bat samples for SARSr-CoVs**

Description and execution:

Preliminary Data:

Organization leading task: Wuhan Institute of Virology, Duke-NUS

Progress Metrics:

Deliverable(s):

***(TA1)* Subtask 3.1: Characterize and isolate high-risk SARSr-CoV quasispecies**

Description and execution:

Preliminary Data:

Organization leading task: Wuhan Institute of Virology, Duke-NUS

Progress Metrics:

Deliverable(s):

(TA1) **Task 4: Develop recombinant chimeric spike proteins from characterized SARSr-CoVs**

Description and execution:

Preliminary Data:

Organization leading task: University of North Carolina

Progress Metrics:

Deliverable(s):

(TA2) **Task 5: Trial experimental approaches aimed towards 'Broadscale Immune Boosting' using experimental bat colonies**

Description and execution:

Preliminary Data:

Organization leading task: Wuhan Institute of Virology, Duke-NUS

Progress Metrics:

Deliverable(s):

(TA2) **Task 6: Trial experimental approaches aimed towards 'Immune Targeting' using experimental bat colonies**

Description and execution:

Preliminary Data:

Organization leading task: University of North Carolina

Progress Metrics:

Deliverable(s):

(TA2) **Task 7: Develop and assess delivery methods for immune boosting and priming molecules**

Description and execution:

Preliminary Data:

Organization leading task: USGS National Wildlife Health Center

Progress Metrics:

Deliverable(s):

(TA1) **Task 8: Construct genotype-to-phenotype machine learning models to predict risk of viral evolution and spillover**

Description and execution:

Preliminary Data:

Organization leading task: EcoHealth Alliance

Progress Metrics:

Deliverable(s):

Phase II:

(TA1) **Task 9: Design LIPS assays to specific high- and low- zoonotic risk SARSr-CoVs**

Description and execution:

Preliminary Data:

Organization leading task: Wuhan Institute of Virology, Duke-NUS

Progress Metrics:

Deliverable(s):

(TA1) **Task 10: Validate model predictions of viral spillover risk using data from LIPS**

assays and banked human sera

Description and execution:

Preliminary Data:

Organization leading task: EcoHealth Alliance

Progress Metrics:

Deliverable(s):

***(TA1)* Task 11: Test efficacy of intervention approaches on wild-caught Rhinolophus bats**

Description and execution:

Preliminary Data:

Organization leading task: Wuhan Institute of Virology, Duke-NUS

Progress Metrics:

Deliverable(s):

***(TA1)* Task 12: Construct models to maximize efficacy of intervention approaches**

Description and execution:

Preliminary Data:

Organization leading task: EcoHealth Alliance

Progress Metrics:

Deliverable(s):

***(TA2)* Task 13: Deploy most effective molecule delivery methods on bat colonies of Yunnan Province caves**

Description and execution:

Preliminary Data:

Organization leading task: EcoHealth Alliance

Progress Metrics:

Deliverable(s):

H) Schedule and Milestones

- Provide a detailed schedule showing tasks (task name, duration, work breakdown structure element as applicable, performing organization), milestones, and the interrelationships among tasks.

  NOTE: Task structure must be consistent with that in the SOW.

- Measurable milestones should be clearly articulated and defined in time relative to the start of the project.

I) PREEMPT Transition Plan

- Indicate the types of partners (e.g., government, private industry, non-profit)

- Submit a timeline with incremental milestones toward successful engagement. NOTE: begin transition activities during the early stages of the program (Phase I).

- Describe any potential DARPA roles.

J) PREEMPT Risk Mitigation Plan

- Provide the following:

  - An assessment of potential risks to public health, agriculture, plants, animals, the environment, and national security.

  - Guidelines the proposer will follow to ensure maximal biosafety and biosecurity.

  - A communication plan that addresses content, timing, and the extent of distribution of potentially sensitive dual-use information. The plan must also address how input from DARPA, other government, and community stakeholders will be taken into account in decisions regarding communication and publication of potentially sensitive dual-use information.

K) Ethical, Legal, Societal Implications (ELSI)

- Address potential ethical, legal, and societal implications of the proposed technology.

Commented [EA4]: Description from the BAA:

PREEMPT Transition Plan
Proposers must include a PREEMPT Technology Transition Plan. Proposers must indicate the types of partners (e.g., government, private industry, non-profit) they plan to pursue and submit a timeline with incremental milestones toward successful engagement. Proposers should begin transition activities during the early stages of the program (Phase I). Awardees must include
DARPA in the development of transition relationships. If the transition plan includes a start-up company, a business development strategy must be included as well. The extent by which the proposed intellectual property (IP) rights will impede the Government's ability to transition the technology will be considered in the proposal evaluation.

Section III – Additional Information (doesn't count against 36 pg. limit)

A) Brief Bibliography (no page limit indicated – can be published/unpublished)
B) Up to 3 relevant papers attached (optional)

回复: RE: For our DARPA PREEMPT conversations this week: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status

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Thu 3/1/2018 12:19 AM

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Great! Thanks.

SHI Zhengli, Ph. D
Senior Scientist & Professor
Wuhan Institute of Virology, Chinese Academy of Sciences
44 Xiao Hong Shan
430071 Wuhan, Hubei
China
Tel & Fax: (0086) 27 87197240
Email: zlshi@wh.iov.cn

发件人： Peter Daszak
发送时间： 2018-03-01 14:00
收件人： Zhengli Shi (zlshi@wh.iov.cn); Ralph Baric (rbaric@email.unc.edu); 周鹏 (peng.zhou@wh.iov.cn); Wang Linfa; Rocke, Tonie
抄送： Danielle Anderson (danielle.anderson@duke-nus.edu.sg); aaron.irving@duke-nus.edu.sg; Baric, Toni C; sims0018@email.unc.edu; Luke Hamel; William B. Karesh
主题： RE: For our DARPA PREEMPT conversations this week: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status

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Cheers,

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President

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Cc: (b) (6)
Subject: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status

(b) (6)

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BAA Coordinator
Contractor Support to DARPA/BTO
PREEMPT@darpa.mil

RE: For our DARPA PREEMPT conversations this week: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status

Wang Linfa <linfa.wang@duke-nus.edu.sg>

Thu 3/1/2018 12:25 AM

To: Peter Daszak <daszak@ecohealthalliance.org>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Rocke, Tonie E <trocke@usgs.gov>
Cc: Danielle Anderson <danielle.anderson@duke-nus.edu.sg>; Aaron Trent Irving <aaron.irving@duke-nus.edu.sg>; Baric, Toni C <antoinette_baric@med.unc.edu>; sims0018@email.unc.edu <sims0018@email.unc.edu>; Luke Hamel <hamel@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>

Thanks

Will start work on this from weekend. In London for the WHO NiV meeting now.

LF

Linfa (Lin-Fa) WANG, PhD FTSE
Professor & Director
Programme in Emerging Infectious Disease
Duke-NUS Medical School,
8 College Road, Singapore 169857
Tel: +65 6516 8397

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Thursday, 1 March, 2018 2:01 PM
To: Zhengli Shi (zlshi@wh.iov.cn); Ralph Baric (rbaric@email.unc.edu); 周鹏 (peng.zhou@wh.iov.cn); Wang Linfa; Rocke, Tonie
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RE: For our DARPA PREEMPT conversations this week: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status

Baric, Ralph S <rbaric@email.unc.edu>

Fri 3/2/2018 10:38 AM

To: Peter Daszak <daszak@ecohealthalliance.org>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; Rocke, Tonie E <trocke@usgs.gov>; Sheahan, Timothy Patrick <sheahan@email.unc.edu>
Cc: Danielle Anderson (danielle.anderson@duke-nus.edu.sg) <danielle.anderson@duke-nus.edu.sg>; aaron.irving@duke-nus.edu.sg <aaron.irving@duke-nus.edu.sg>; Baric, Toni C <antoinette_baric@med.unc.edu>; Sims, Amy C <sims0018@email.unc.edu>; Luke Hamel <hamel@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>

I have included Tim in the email chain. Ralph

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Thursday, March 1, 2018 1:01 AM
To: Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Baric, Ralph S <rbaric@email.unc.edu>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; Rocke, Tonie <trocke@usgs.gov>
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Contractor Support to DARPA/BTO
PREEMPT@darpa.mil

PREEMPT meeting notes (Week of 26 Feb - 2 Mar)

Luke Hamel <hamel@ecohealthalliance.org>

Fri 3/2/2018 8:44 PM

To: Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; wang linfa <linfa.wang@duke-nus.edu.sg>; Aaron Trent Irving <aaron.irving@duke-nus.edu.sg>; danielle.anderson@duke-nus.edu.sg <danielle.anderson@duke-nus.edu.sg>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Sims, Amy C <sims0018@email.unc.edu>; Rocke, Tonie E <trocke@usgs.gov>; Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; William B. Karesh <karesh@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Alice Latinne <latinne@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Carlos Zambrana-Torrelio <zambrana@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>; sheahan@email.unc.edu <sheahan@email.unc.edu>
Cc: Dr. Peter Daszak <daszak@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Baric, Toni C <antoinette_baric@med.unc.edu>; Jonathon Musser <musser@ecohealthalliance.org>

Hi all,

I wanted to provide you with the **latest notes from various PREEMPT calls** that took place this week (listed below). Meeting notes from the call between EHA and Wuhan + Duke-NUS are the same as those sent earlier this week.

1) Tue. 27 Feb. (Call between **EHA** staff and staff from **Wuhan + Duke-NUS**)
2) Fri. 2 March (Call between **Peter** and **Jim Gimlett from DARPA**)
3) Fri. 2 March (Call between **EHA** staff and **Ralph + Tim @ UNC**)
4) Fri. 2 March (Call between **EHA** staff and **Tonie @ USGS NWHC**)

Below, I've listed the 'Action Items' that correspond to each call. These action items are also listed at the top of each document

Call between EHA & Wuhan/Duke-NUS

****Action Items:**

- PD to have weekly calls w/ collaborators
  - LH to organize these calls
- LH to send an email confirming: DUNS nos., addresses and phone numbers of each collaborating institution.
  - The email will also provide instructions for how to sign up on Grants.gov (required for Key Personnel of each collaborating organization)
- PD to sit down with KJO, JHE and LH to determine who will be responsible for each section of the proposal. Will send to collaborators by **Thu. 3/1**
  - Collaborators to then write their respective sections and return to PD by **Wed. 3/7**
  - Peng to include section about how both Immune Boosting and Immune Targeting approaches are better than vaccination (without explicitly criticizing vaccination approach)
- PD to speak with YunZhi regarding consultant field work in/around Yunnan cave
- Collaborators to determine if any additional personnel required for their work (e.g. technicians, field support staff, etc.)
  - Collaborators to let PD know if they think anyone else should be brought on to the team

-PD to determine which aspects of full proposal are baseline tasks, and which are add-ons **(by mid to late March)**

Call between EHA & Ralph/Tim (UNC)

****Action Items:**

- RB and team to draft their section and return to PD by **Wed. 3/7**
  - RB to include section stating how our project won't drive viral evolution in negative way
- PD to complete quality first draft by end of next week, **Fri. 3/9**
- Jonathon Musser (EHA) and Amy Sims (UNC) to work together on UNC budget

-Once EHA has drafted modeling section of proposal, RB to provide input

Call between EHA & Tonie (NWHC)

**Action Items:

- Tonie Rocke (TR) to begin work on her section of the full proposal (and send to PD by **Wed. 3/7**)
  - TR to cite literature referring to use of transdermal vaccine application
- TR to begin drafting budget for NWHC

- TR to check with folks from F&WS to learn about their work using automatic sprayers for WNS

As always, please let me know if you have any questions.

Best,

Luke Hamel

Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

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PREEMPT call (Peter, Jim Gimlett of DARPA) - 2 March 2018

- **Re: Issue of human subjects/samples**
  - For the full proposal, we have to explicitly state that any human samples we use will only come from previous studies, and that we have full access to these samples
    - PREEMPT will NOT fund any human sampling efforts
  - When referencing previously collected human samples (which we could have 1000s of), mention how we will triage samples to determine priority for testing, as well as how we plan to ship samples out

- **Re: Number and size of DARPA awards**
  - According to Jim Gimlett (Program Manager for PREEMPT), DARPA has received many promising abstracts, and likely won't be able to fund all of them (or at least won't be able to fund every aspect of each project)
    - Jim is contacting other DARPA personnel to try and get additional funding for later stages of accepted proposals
      - In the meantime, it's important that we separate tasks/subtasks into 'baseline' tasks and 'add-on' tasks (as add-ons may be cut from proposed projects)

- **Re: EHA collaboration with Wuhan**
  - PD mentioned our existing collaboration with Wuhan, to Jim.
    - PD mentioned our standard operating procedure, and that our collaboration with Wuhan involves complete access to information and leads to research with quickly produced results
  - Jim checked in with higher ups at DARPA, and they've mentioned that there are no current political issues about us working in China
    - This is great news as we'll be able to move ahead with our proposal as planned
    - Also, the fact that Jim inquired about our collaboration with Wuhan is a good sign that he's interested in our proposal and wants to fund it.

- **Re: Jim Gimlett's response about riskiness of our proposal**
  - PD asked Jim if anything about our proposal seemed too risky, or not risky enough
    - Jim didn't have anything to say specifically, so we should be on the right track.
      - He did mention, however, that we should show how we'll validate everything

        - i.e. Show that immune modulation works at every step, and show validation through modeling approaches

- **Re: Concerns about host response to inocula**
    - One DARPA employee mentioned the possibility of bats reacting negatively to our inocula.
        - So we MUST show preliminary data when we write about this in our proposal
            - Show how our team has demonstrated the effectiveness of our proposed intervention approaches - how we can reduce viral load by upregulating the immune response
                - Provide additional detail for interferon work specifically

- **Some additional comments from Billy Karesh (BK):**
    - The modeling approaches that we list in the proposal must be tied directly to what the lab and field work are attempting to accomplish. In other words, we can't just expand our modeling efforts in order to address questions from the data that we find interesting.
        - Rather, our modeling must be 'utilitarian'. Modeling is strictly to inform our lab and field teams on how to create a more effective intervention approach (e.g. Use modeling to predict which high-risk viral strains to target in the lab)

PREEMPT call (EHA, Ralph + Time of UNC) - 2 March 2018

****Action Items:**

- RB and team to draft their section and return to PD by **Wed. 3/7**
  - RB to include section stating how our project won't drive viral evolution in negative way
- PD to complete quality first draft by end of next week, **Fri. 3/9**
- Jonathon Musser (EHA) and Amy Sims (UNC) to work together on UNC budget
- Once EHA has drafted modeling section of proposal, RB to provide input

- **Re: Ralph Baric and team**
  - Tim Sheahan (Research assistant professor at UNC)
    - Tremendous amount of experience working w/ CoV reverse genetics/ synthetic reconstruction of CoVs
    - Genetics expert. Has been looking into Lineage D BetaCoVs, in terms of trying to find strains that can replicate in human cells
  - Amy Sims (Research associate professor)
    - Great deal of experience working w/ primary human cells and artificial construction of SARSr-CoVs to test binding to human cells. Lots of drug testing + vaccine work against SARS and MERS
    - Can also help with the subaward budget.

- **Re: UNC subcontract budget**
  - It will take roughly 7-10 days for RB and his team to review/get approval for the budget.
  - Jonathon Musser (EHA) and Amy Sims (UNC) to work together on UNC budget

- **Re: Modeling approaches**
  - Noam Ross (NR) would like to be able to look at inputs of both sequence data as well as ecological/host data for viruses, and make predictions about which virus traits we see when they're in infected mice, as well as the degree to which they show up in nearby human populations
    - To understand this, it'd be helpful to know how many different viral strains (and of what risk), we'd put through the humanized mouse process in order to get an understanding of their ability to infect mice (as well as what the viral growth in those models are).
      - NR interested to hear Ralph Baric's (RB) thoughts on how many different strains we'd test over the course of project. A small handful? Dozens?
    - Ralph says it's very budget dependent. We could look at pseudotyped viruses to see which spikes drive entry.

Commented [1]: Ralph/Tim- Please confirm this

- For SARSr-CoVs, we know there are strains ranging from epidemic strains to those w/ 10-12% variation in spike protein, that still can infect human cells and humanized mice.
    - Within this 10-12% range, RB has tested one strain w/ 8% variation and one w/ 3%.
        - So the goal when looking for strains w/ novel variance, is to look for those w/ relatively conserved receptor binding domains (RBDs) but other variation of the spike that's w/in the 10-12% window. 2 or 3 strains perhaps.
        - Once you have strains with greater spike variation (10-20%), there's a reduction in capacity of viruses to grow in human cells or use human ACE2 receptor (unless RBD is conserved).
            - Through recombination, it's possible that the correct RBD could be dropped into a strain w/ 25% variation, and allow the virus to enter human cell and replicate
    - Given a 42-month time period, we'd probably screen 15-16 viruses to determine which strains we'd like to focus in on (given spike proteins), likely leaving us with 7 or 8.

- Peter (PD) thinks Noam's modeling could involve:
    - An initial prediction of virus binding to ACE2 receptor
    - Then whether virus can replicate in human cells
    - Then focus on humanized mice and whether disease is caused in humanized mice that looks like SARSr-CoV (This is gold standard in the end)

- Ralph thinks there are probably 5 viruses to be used as baselines - viruses that we know can replicate and use human receptor (SARS, Raccoon dog isolate, WIV16, WIV1, SCH014). These 5 capture range of 1% -12% variation in spike.
    - If you look at WIV16, WIV1 and SCH014, the RBDs that engage the receptor are fairly well conserved and can be modeled.
        - Want to model RBD, ACE2 interaction (3D protein modeling)
            - RB has people who can help w/ this. Ultimately, we're looking for strains that conserve some sort of RBD integrity. We're not interested in strains w/ loss of contact interface sites or deletion of RBD.
                - Knowing this will help guide modeling efforts

    - We also need to model using information on <u>protease cleavage site</u>.
        - For SARSr-CoVs, there's a two-step entry process.
            - 1) Virus-receptor interaction

            - 2) Either extracellular protease or Intracellular protease have to clip protein once or twice so that virus can fuse to cell membrane
                - Virus can't enter cell w/o protease. In the lab, if protease is added exogenously, strains that otherwise would not enter cell are now able

    - So these are the 2 major modeling processes (the 2 screening components)
        - You screen (1) on the spike and (2) on the protease
    - Some steps that RB says modeling could incorporate:
        - (1) Very conserved RBD
        - (2) Sequence variance around RBD
        - (3) Protease cleavage site
        - (4) What RBD looks like in relation to conformity to binding sites
        - (5) Overall spike variation
        - (6) RNA recombinance (maybe RBD that is capable of interfacing w/ human receptor, but has very different genetic background of the whole spike)
    - Model these to show progressively lower risk that virus could bind to and infect cells)

- NR knows that in terms of the viruses being tested/screened, there's a small set taken all the way to humanized mice.
    - But in terms of screening assays, are we conducting one assay to screen for spike binding, and another to screen for protease? In other words, are we testing the 2 components independently, and then based on the success of both, making a decision of how to move forward to test in our model?
        - Ralph says that the easiest way to approach this, is either w/ pseudotyped viruses or chimera viruses w/ full length spike.
            - First look at receptor binding on cells, then look at virus ability to get into cells (remembering that getting into cell requires protease).
                - You'll have spikes that bind to surface but can't get in, and the basic approach here is to add exogenous protease to chimera virus system or pseudotyped virus to see if it can get in

- Need to make sure that RB reads the EHA modeling approach to make sure we have the right approach.
    - What we need to do is determine exactly what NR will model
        - Ralph thinks modeling is 5-step process
            - (1) Find where viral sequence fits within phylogenetic tree, especially in relationship to 5 baseline viruses (SARS, raccoon dog isolate, WIV1, WIV16, SCH014

- (2) Focus on RBDs and models that predict whether viruses interact w/ mouse or human ACE2 molecules (Human would be priority, but could do mouse)
- (3) From those models, determine:
    - Where is variation?
    - How many contact residues retained?
    - Are there deletions?
        - If so, these strains get deprioritized
- (4) Look for protease cleavage sites in strains w/ SARS like spikes
    - Are sequence signatures present?
        - If yes, that's a plus
- (5) Look at overall spike variation (recombinant molecule that's picked up favorable RBD and dropped into variable spike protein)
    - This will predict which 8-16 strains we'll synthesize
        - $1000/spike. So $16k?

- **DARPA wants machine learning (ML) to be incorporated**. They want some cool modeling approach that tells you the following:
    - When a warfighter goes to a region…
        - 1) Is it a place w/ Ebola and/or other high-risk strains that spill into humans?
        - 2) Can we predict which species carry these viruses?
    - So we just need to make sure we include the following:
        - The species of bats that are likely to harbor viral strains very similar to SARS virus
        - The species assemblages that are likely to drive evolution of those strains.
    - Then all other stuff comes in when we try to validate models. We don't need super technical details now. DARPA just wants to make sure we have preliminary data to do the work, and that later on we can validate models and immune modulation approaches.
        - This shouldn't be a problem as we all have preliminary data (RB, Linfa/Zhengli, TR, EHA)

- So RB thinks it will be reiterative process.
    - First look at sequences, prioritize the top 4, then test them and use the data to refine models (perhaps using ML)
    - Then reassess the top candidates, then do another 4 (i.e. stepwise modeling), then take 5 or 6 criterium and figure out how to prioritize which ones weight most heavily on predictive success
        - NR agrees but thinks scale is a challenge

- Due to fact that statistical strength will come from linking viral sequence to its success binding and infecting cells, we won't really have a large dataset to work with.
    - RB says there must be 100+ spike protein variants that have been sequenced (at least). We also know rough boundaries that determine virus potential to replicate efficiently and use human receptors. So we can use these boundaries in our models as well.
        - NR thinks that even if we have enough sequences (input), our output (and ability to statistically validate models) still requires data on viruses that can successfully bind to and infect human cells. And we likely won't find that many strains that are successful
            - So we have to lean more heavily on mechanistic approach (emphasizing knowledge of RBD variation into predictive model itself), b/c we have limited set of iterations we can do

- PD says that ultimately, our final proposal is all about utilitarian modeling.
    - i.e. Modeling is strictly to inform our lab and field teams on how to create a more effective intervention approach (e.g. use modeling to predict which high-risk viral strains to target in the lab)
    - Also, we MUST make it clear in proposal that our approach won't drive evolution the wrong way (e.g. drive evolution of more virulent strain that then becomes pandemic
        - This needs to be explained in detail (RB will draft this section)

- **Re: 'Immune Priming' approach**
    - Essentially, the approach is to upregulate both acquired and innate immunity against viral spikes
    - Most juvenile bats have SARSr-CoVs, but titers drop in older adults
        - Use recombinant protein vaccine paired w/ some sort of adjuvant mixture to stimulate innate immunity and knock down titer (this should reduce virus burden in cave)
            - In full proposal, stress that we're designing simple, straightforward approach that plays on bat immunity and offers transient protection from viral spillover

- **Re: Delivery method of inoculum**
    - How will the recombinant spike proteins be administered?

        - RB thinks intranasal administration most effective. Perhaps as aerosolized spray
            - Dextran microparticle that can be formulated either as microparticle-based vaccine or nanoparticle (so could be delivered by spray)
                - The antigen is interlaced inside matrix and can be delivered as spray.
                    - Large particles go directly to macrophage for vaccine delivery. Small ones go to dendritic cells.
            - Never been applied to populations like this.
                - So potentially some risk here
            - Idea is to propose 'Immune Boosting' and 'Immune Priming' methods as concurrent approaches
                - Start both approaches at the beginning of the project, then have friendly competition between the two
                    - Some things will work, some won't. Focus on what works, so by end of project we have spray that warfighter sends into bat cave to transiently reduce risk of spillover
                        - Could be mixture of molecules (Poly IC plus RB's recombinants, intranasal + sticky gel, etc.)

- **Re: PREEMPT Transition Plan, translation plan**
    - DARPA wants us to determine who a potential customer/recipient of our work could be, to take research to next stage or apply it in way that's useful to them
        - Potentially US Army, to use in order to reduce spillover risk to troops
        - DARPA (unlike US Army, DTRA, etc.) isn't an end-user. They fund projects but don't end up using the final product
    - If we're designing treatment, will it be publicly available? Handed over to DoD?
    - RB has some experience with this. Is working on commercial vaccine for flavivirus, and also doing collaborative work for drugs on emerging CoV infection.
    - Some <u>potential translation ideas for our proposal</u>:
        - Panel of new viruses that sets DoD up to prepare for breadth of viruses, allowing for creation of vaccines.
        - Animal models that can be used to evaluate therapeutics.
        - ML programs designed to take specific features in a virus family and do a reiterative prediction about pre-pandemic potential.
            - Model will be CoV but should be able to translate, transition into other systems where you know the surface protein (major species specificity factor) all of these proteases, etc. Also variation in RBD is known.

        - Bats that we'll sequence will provide massive amounts of these variations that can be modeled onto ACE2 bat molecule, to see how virus has caused species to coevolve, or try to escape virus pressure.
            - i.e. interface site of ACE2 moledule in bat will show most variation, b/c virus will put selective pressure on animals, and bats w/ ability to escape, will be ones that are maintained in the population.
                - This will drive variation in RBD and ability to use human receptor.
    - Another idea is...if you build chimera that broadly reduces heterogeneous pop. of SARSr-CoVs in bat cage, this might be something you'd want to develop for humans.
        - RB has already generated SARS-like chimeras w/ RBD from group of bat viruses called 293 (for S1), which is 20% different than epidemic strains, and S2 region from HK3 which is 20% diff.
            - Can drive broad-based rsponese for large no. of family members (but we can test those)
            - **Detailed sequence analysis could be used to engineer broad -based vaccinations for humans**

- **Re: Intentional wording w/in the proposal**
    - In technical sections of full proposal, be careful not to confuse the reviewers (e.g. using 'vaccine', 'immune modulation', 'viral suppression'
        - Just talk about transiently reducing spillover, through modulation of bat immune response
            - Then when talking about translational plan, we can mention potential vaccines for human application
        - We just want to be careful that we're not sounding overly ambitious (e.g. that we'll try 6 different treatment as well as vaccines)
            - <u>Ensure that the terminology we use reflects a project that can be completed in a 42-month period</u>

- **Re: Nanoparticle delivery method**
    - Ensure that RB and TR come together when discussing potential nanoparticle delivery of inocula.

PREEMPT call (EHA, Wuhan, Duke-NUS) - 27 Feb 2018

****Action Items:**

- PD to have weekly calls w/ collaborators
  - LH to organize these calls
- LH to send an email confirming: DUNS nos., addresses and phone numbers of each collaborating institution.
  - The email will also provide instructions for how to sign up on Grants.gov (required for Key Personnel of each collaborating organization)
- PD to sit down with KJO, JHE and LH to determine who will be responsible for each section of the proposal. Will send to collaborators by **Thu. 3/1**
  - Collaborators to then write their respective sections and return to PD by **Wed. 3/7**
  - Peng to include section about how both Immune Boosting and Immune Targeting approaches are better than vaccination (without explicitly criticizing vaccination approach)
- PD to speak with YunZhi regarding consultant field work in/around Yunnan cave
- Collaborators to determine if any additional personnel required for their work (e.g. technicians, field support staff, etc.)
  - Collaborators to let PD know if they think anyone else should be brought on to the team
- PD to determine which aspects of full proposal are baseline tasks, and which are add-ons **(by mid to late March)**

- **Re: Timeline**
  - PD sends collaborators full proposal template with assigned sections, by **Thu. 3/1**
  - Collaborators complete their sections and return to PD by **Wed. 3/7**
  - Quality first draft of full proposal, completed by **Mon. 3/12**
  - Send out draft budget to collaborators by **Mon. 3/19 at latest**
    - Budget turnarounds for each collaborating institution:
      - Duke-NUS: 1-2 days
      - Wuhan Univ.: 1-2 days
      - UNC (Ralph): PD to ask on call
      - NWHC (Tonie): PD to ask on call
  - By **mid to late March**, PD is going to go thru proposal, thinking carefully about timeline and budget. He'll make sure each collaborator is doing both baseline work and additional work

- **Re: Writing the full proposal**
  - PD will work with KJO, JHE and LH to determine which section of the proposal each collaborator will write

        - The plan is for each group to work on the technical aspects they're experts on, rather than PD writing a draft of each section first
    - By **Thu. 3/1**, PD to send around the proposal w/ names attached to which parts we need flushing out.
    - Then, over 5-day period, collaborators can draft out their sections and return to PD **by Wed. 3/7**
        - PD would rather have more detailed, technical language than less. PD has no problem cutting down info.
            - For each task/subtask, provide any preliminary data (e.g. no. of caves we've visited, no. of samples we've collected, data describing our work w/ Rhinolophus, etc.).
                - Include any relevant references, numbers, figures, charts
        - Please use EndNote for references, or send references as text (can be fixed later)
        - When writing your section(s), don't separate tasks into baseline tasks vs. add-ons (PD will decide this)

- **Re: The proposed budget**
    - DARPA to fund 0-6 projects ($40 million total)
    - We have to be careful and ensure we've justified the money we're asking for
        - We need to distinguish between 'baseline' tasks and 'add-ons'
            - This will give DARPA clear tasks to cut if funding is limited
            - Given that we have a strong team, the goal is to make sure that each collaborator is responsible for both baseline tasks and add-ons
                - This way if our proposed budget is reduced, each collaborator is still able to contribute to the project
            - We may need to reconsider carrying out lab work at 4 different collaborator institutions.
                - DARPA might not fund lab work at all 4
            - **By mid to late March**, PD is going to go thru proposal, thinking carefully about timeline and budget. He'll make sure each collaborator is doing both baseline work and additional work

- **Re: Distinguishing our approach from vaccination approach**
    - Ralph Baric's approach is not vaccination. Rather his approach aims to boost the immune response against specific viral proteins (potentially to include innate immune response)
        - Vaccination in bats not likely to work.
            - Very limited antibodies from natural viral infection in bats
            - Viruses already common in bat population - not likely to challenge bat immune response with a vaccine

- Peng to write section about how both Immune Boosting and Immune Targeting approaches are better than vaccination (without explicitly criticizing vaccination approach)
    - Our aim is to show DARPA that we will...Develop and test a technology that's not guaranteed to work (but is ambitious and has great potential) that allows us to reduce risk of spillover (even if only temporarily)

Re: PREEMPT meeting notes (Week of 26 Feb - 2 Mar)

Luke Hamel <hamel@ecohealthalliance.org>

Tue 3/6/2018 2:35 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Jonathon Musser <musser@ecohealthalliance.org>

Hi Tonie,

Friday will be fine - we hope you feel better! Also, I am working with Jonathon Musser of EHA (cc'd here) to create a budget template that we will send to you.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Tue, Mar 6, 2018 at 10:20 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Luke: Can you please send me your projected budget for my task? Also, I thought we had agreed to a final draft due on Friday, 3/9, not Wednesday. Full disclosure, I have contracted the flu and I'm pretty out of it. I've been working on it, but I probably won't get my draft done until Friday, Thanks -Tonie
>
> On Fri, Mar 2, 2018 at 8:44 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi all,
> >
> > I wanted to provide you with the **latest notes from various PREEMPT calls** that took place this week (listed below). Meeting notes from the call between EHA and Wuhan + Duke-NUS are the same as those sent earlier this week.
> >
> > 1) Tue. 27 Feb. (Call between **EHA** staff and staff from **Wuhan + Duke-NUS**)
> > 2) Fri. 2 March (Call between **Peter** and **Jim Gimlett from DARPA**)
> > 3) Fri. 2 March (Call between **EHA** staff and **Ralph + Tim @ UNC**)

4) Fri. 2 March (Call between **EHA** staff and **Tonie @ USGS NWHC**)

Below, I've listed the 'Action Items' that correspond to each call. These action items are also listed at the top of each document

Call between EHA & Wuhan/Duke-NUS

****Action Items:**

- PD to have weekly calls w/ collaborators
  - LH to organize these calls
- LH to send an email confirming: DUNS nos., addresses and phone numbers of each collaborating institution.
  - The email will also provide instructions for how to sign up on Grants.gov (required for Key Personnel of each collaborating organization)
- PD to sit down with KJO, JHE and LH to determine who will be responsible for each section of the proposal. Will send to collaborators by **Thu. 3/1**
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- PD to speak with YunZhi regarding consultant field work in/around Yunnan cave
- Collaborators to determine if any additional personnel required for their work (e.g. technicians, field support staff, etc.)
  - Collaborators to let PD know if they think anyone else should be brought on to the team

-PD to determine which aspects of full proposal are baseline tasks, and which are add-ons **(by mid to late March)**

Call between EHA & Ralph/Tim (UNC)

****Action Items:**

- RB and team to draft their section and return to PD by **Wed. 3/7**
  - RB to include section stating how our project won't drive viral evolution in negative way
- PD to complete quality first draft by end of next week, **Fri. 3/9**
- Jonathon Musser (EHA) and Amy Sims (UNC) to work together on UNC budget

-Once EHA has drafted modeling section of proposal, RB to provide input

Call between EHA & Tonie (NWHC)

**Action Items:

- Tonie Rocke (TR) to begin work on her section of the full proposal (and send to PD by **Wed. 3/7**)
  - TR to cite literature referring to use of transdermal vaccine application
- TR to begin drafting budget for NWHC

- TR to check with folks from F&WS to learn about their work using automatic sprayers for WNS

As always, please let me know if you have any questions.

Best,

Luke Hamel
Program Assistant

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--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

Scheduling upcoming calls with Peter (PREEMPT)

Luke Hamel <hamel@ecohealthalliance.org>

Tue 3/6/2018 2:38 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Dr. Peter Daszak <daszak@ecohealthalliance.org>

Hi Tonie,

Peter is hoping to arrange weekly calls with you, to ensure that we stay on track with the full proposal.

Please use this link to select the date(s)/time(s) you are available to speak with Peter (select as many as you are available for). The goal is to have one call each week, on either Thursday or Friday. Below, I've listed the dates/times that appear in the Doodle Poll link above. Please note that all times are in Eastern Time

Week 1:
Thu. 3/8 (11 AM - 5 PM ET)
Thu. 3/9 (11 AM - 5 PM ET)

Week 2:
Thu. 3/15 (11 AM - 5 PM ET)
Fri. 3/16 (11 AM - 5 PM ET)

Week 3:
Thu. 3/22 (11 AM - 5 PM ET)
Fri. 3/23 (11 AM - 5 PM ET)

Please let me know if you have any questions.

Best,

Luke Hamel
Program Assistant

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Re: Scheduling upcoming calls with Peter (PREEMPT)

Luke Hamel <hamel@ecohealthalliance.org>

Wed 3/7/2018 1:32 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Dr. Peter Daszak <daszak@ecohealthalliance.org>

Thank you for letting me know, Tonie. Hope you're feeling better today!

Best,

Luke Hamel
Program Assistant

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On Wed, Mar 7, 2018 at 2:24 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Luke: I signed up for calls this Friday and next, but I will be out of the country on Mar 22-23. I am available instead on Wednesday March 21. -Tonie
>
> On Tue, Mar 6, 2018 at 2:38 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > Peter is hoping to arrange weekly calls with you, to ensure that we stay on track with the full proposal.
> >
> > **Please use this link to select the date(s)/time(s) you are available to speak with Peter (select as many as you are available for)**. The goal is to have one call each week, on either Thursday or Friday. Below, I've listed the dates/times that appear in the Doodle Poll link above. Please note that all times are in Eastern Time
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Fri. 3/16 (11 AM - 5 PM ET)

Week 3:
Thu. 3/22 (11 AM - 5 PM ET)
Fri. 3/23 (11 AM - 5 PM ET)

Please let me know if you have any questions.

Best,

Luke Hamel
Program Assistant

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--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

PREEMPT budget template and details

Luke Hamel <hamel@ecohealthalliance.org>

Wed 3/7/2018 4:03 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Dr. Peter Daszak <daszak@ecohealthalliance.org>

Hi Tonie,

Please see attached, a **PREEMPT budget template** for you to complete. Although this template lacks specific detail that will be required later, it will allow both you and our staff at EHA to determine a general budget breakdown for your institution.

At the moment, please focus your attention on sections A-K, and fill out these sections as completely as possible. Section L (Budget justification) can be addressed at a later time, and I will be sure to provide you with additional instructions within the upcoming week.

To assist you in completing the budget template, I have included the proposed disbursement schedule for the EHA-NWHC subcontract.

Year	Amount
Y1	$304,500.00
Y2	$304,500.00
OY1	$304,500.00
OY .5	$152,250.00
Total	**$1,065,750.00**

Lastly, please keep in mind the following items as you begin to determine specific aspects of your budget. Although this level of detail isn't needed for you to complete the attached budget template, it will be required for the final budget submission.

Please let me know if you have any questions**. EHA staff will gladly assist you in procuring the necessary items, as required below.**

1) Regarding 'Indirect Costs':
If available, provide current Forward Pricing Rate Agreement or Forward Pricing Rate Proposal. If not available, provide 2 years historical data to include pool and expense costs used to generate the rates. For academia, provide DHHS or ONR negotiated rate package, or, if calculated by other than a rate, provide University documentation identifying G&A and fringe costs by position.

2) Regarding 'Travel':
Provide the purpose of the trip, number of trips, number of days per trip, departure and arrival destinations, number of people, estimated rental car and airfare costs, and prevailing per diem rates as determined by gsa.gov, etc.; Quotes must be supported by screenshots from travel websites.

3) Regarding 'Equipment Purchases'
Itemization with individual and total costs, including quantities, unit prices, proposed vendors (if known), and the basis of estimate (e.g., quotes, prior purchases, catalog price lists, etc.); any item that exceeds $5,000 must be supported with back-up documentation such as a copy of catalog price lists or quotes prior to purchase (NOTE: For equipment purchases, include a letter stating why the proposer cannot provide the requested resources from its own funding.

4) Regarding 'Materials'
Itemization with costs, including quantities, unit prices, proposed vendors (if known), and the basis of estimate (e.g., quotes, prior purchases, catalog price lists, etc.); any item that exceeds $5,000 must be supported with back-up documentation such as a copy of catalog price lists or quotes prior to purchase.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(b) (6) (mobile)
www.ecohealthalliance.org

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WORKSPACE FORM

1-800-518-4726
SUPPORT@GRANTS.GOV

This Workspace form is one of the forms you need to complete prior to submitting your Application Package. This form can be completed in its entirety offline using Adobe Reader. You can save your form by clicking the "Save" button and see any errors by clicking the “Check For Errors” button. In-progress and completed forms can be uploaded at any time to Grants.gov using the Workspace feature.

When you open a form, required fields are highlighted in yellow with a red border. Optional fields and completed fields are displayed in white. If you enter invalid or incomplete information in a field, you will receive an error message. Additional instructions and FAQs about the Application Package can be found in the Grants.gov Applicants tab.

OPPORTUNITY & PACKAGE DETAILS:

Opportunity Number:	HR001118S0017
Opportunity Title:	PREventing EMerging Pathogenic Threats
Opportunity Package ID:	PKG00237724
CFDA Number:	12.910
CFDA Description:	Research and Technology Development
Competition ID:	
Competition Title:	
Opening Date:	01/19/2018
Closing Date:	03/27/2018
Agency:	DARPA - Biological Technologies Office
Contact Information:	BAA Coordinator PREEMPT@darpa.mil

APPLICANT & WORKSPACE DETAILS:

Workspace ID:	WS00094394
Application Filing Name:	Project DEFUSE
DUNS:	0770900660000
Organization:	ECOHEALTH ALLIANCE INC.
Form Name:	R & R Subaward Budget 10 YR Subform
Form Version:	1.4
Subform Name:	USGS Ntl. Wildlife Health Cen
Requirement:	Optional
Download Date/Time:	Mar 06, 2018 05:28:38 PM EST
Form State:	Error(s)

FORM ACTIONS:

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 1** **Start Date:** **End Date:**

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Tonie		Rocke								

Project Role: Co-Investigator

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						

Total Number Other Personnel

Total Other Personnel

Total Salary, Wages and Fringe Benefits (A+B)

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.		
9.		
10.		
	Total Other Direct Costs	

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)

Total Indirect Costs

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	

L. Budget Justification

(Only attach one file.) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		
1. Materials and Supplies		
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		
Section H, Indirect Costs		
Section I, Total Direct and Indirect Costs (G + H)		
Section J, Fee		
Section K, Total Costs and Fee (I + J)		

Re: Scheduling upcoming calls with Peter (PREEMPT)

Luke Hamel <hamel@ecohealthalliance.org>

Wed 3/7/2018 4:14 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Dr. Peter Daszak <daszak@ecohealthalliance.org>

Hi Tonie,

Peter won't have cellphone reception on Fri., 3/9, so we are looking to reschedule our call to next week, either Tuesday (3/13) or Wednesday (3/14). I will send out another Doodle Poll link within the week, so please be on the lookout for that email.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
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New York, NY 10001

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On Wed, Mar 7, 2018 at 2:32 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Thank you for letting me know, Tonie. Hope you're feeling better today!
>
> Best,
>
> **Luke Hamel**
> *Program Assistant*
>
> EcoHealth Alliance
> 460 West 34th Street – 17th floor
> New York, NY 10001
>
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> (b) (6) (mobile)
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On Wed, Mar 7, 2018 at 2:24 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Luke: I signed up for calls this Friday and next, but I will be out of the country on Mar 22-23. I am available instead on Wednesday March 21. -Tonie
>
> On Tue, Mar 6, 2018 at 2:38 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > Peter is hoping to arrange weekly calls with you, to ensure that we stay on track with the full proposal.
> >
> > **Please use this link to select the date(s)/time(s) you are available to speak with Peter (select as many as you are available for)**. The goal is to have one call each week, on either Thursday or Friday. Below, I've listed the dates/times that appear in the Doodle Poll link above. Please note that all times are in Eastern Time
> >
> > Week 1:
> > Thu. 3/8 (11 AM - 5 PM ET)
> > Thu. 3/9 (11 AM - 5 PM ET)
> >
> > Week 2:
> > Thu. 3/15 (11 AM - 5 PM ET)
> > Fri. 3/16 (11 AM - 5 PM ET)
> >
> > Week 3:
> > Thu. 3/22 (11 AM - 5 PM ET)
> > Fri. 3/23 (11 AM - 5 PM ET)
> >
> > Please let me know if you have any questions.
> >
> > Best,
> >
> > **Luke Hamel**
> > *Program Assistant*
> >
> > EcoHealth Alliance
> > 460 West 34th Street – 17th floor
> > New York, NY 10001
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--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

Confirming dates for PREEMPT calls

Luke Hamel <hamel@ecohealthalliance.org>

Fri 3/9/2018 4:21 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Dr. Peter Daszak <daszak@ecohealthalliance.org>

Hi Tonie,

Based on the availability that you and Peter have listed, I've scheduled calls for the following dates:

Wed. 3/14 @ 10 AM ET
Wed. 3/21 @ 10 AM ET

Please use the domestic conference line for each call (details below).

Phone: 1-719-785-9461
Password: 9784#

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(b) (6) (mobile)
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Re: PREEMPT budget template and details

Luke Hamel <hamel@ecohealthalliance.org>

Fri 3/9/2018 6:03 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Dr. Peter Daszak <daszak@ecohealthalliance.org>

Hi Tonie,

Other than the existing salary restrictions set by USGS NWHC, there are no additional restrictions listed within the PREEMPT Broad Agency Announcement (BAA).

Please keep in mind, however, the following requirement for all government agencies:

"Government entities must clearly demonstrate that the work [they will perform] is not otherwise available from the private sector and provide written documentation citing the specific statutory authority and contractual authority, if relevant, establishing their ability to propose to Government solicitations." **See BAA, page 18**.

Essentially, this means that we will require the following items from you:

1. A document signed by either the NWHC director or by you (on behalf of the NWHC), stating that the NWHC has the 'contractual authority to collaborate/partner/etc. with EcoHealth Alliance in the PREEMPT proposal titled, 'Project DEFUSE.'
2. A statement (within the same document) describing that NWHC is the only public or private sector laboratory with the necessary skills, equipment, resources, etc...to perform the tasks listed within the full proposal. (This language should match, more or less, what you will have already listed within your technical section).

Next week, I will provide you with a template for this document. In the meantime, please communicate this requirement to your director and notify her or him that a signature will be required.

Please let me know if you have any questions.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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On Wed, Mar 7, 2018 at 5:45 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Luke: Sometimes government grants restrict certain expenditures by other government agencies, particularly federal salaries. Do you know if there are any restrictions in this case? Thanks -Tonie
>
> On Wed, Mar 7, 2018 at 4:03 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> Hi Tonie,
>
> Please see attached, a **PREEMPT budget template** for you to complete. Although this template lacks specific detail that will be required later, it will allow both you and our staff at EHA to determine a general budget breakdown for your institution.
>
> At the moment, please focus your attention on sections A-K, and fill out these sections as completely as possible. Section L (Budget justification) can be addressed at a later time, and I will be sure to provide you with additional instructions within the upcoming week.
>
> To assist you in completing the budget template, I have included the proposed disbursement schedule for the EHA-NWHC subcontract.
>
> | Year | Amount |
> |---|---|
> | Y1 | $304,500.00 |
> | Y2 | $304,500.00 |
> | OY1 | $304,500.00 |
> | OY .5 | $152,250.00 |
> | **Total** | **$1,065,750.00** |
>
> Lastly, please keep in mind the following items as you begin to determine specific aspects of your budget. Although this level of detail isn't needed for you to complete the attached budget template, it will be required for the final budget submission.
>
> Please let me know if you have any questions**. EHA staff will gladly assist you in procuring the necessary items, as required below.**
>
> 1) Regarding 'Indirect Costs':
> If available, provide current Forward Pricing Rate Agreement or Forward Pricing Rate Proposal. If not available, provide 2 years historical data to include pool and expense costs used to generate the rates. For academia, provide DHHS or ONR negotiated rate package, or, if calculated by other than a rate, provide University documentation identifying G&A and fringe costs by position.
>
> 2) Regarding 'Travel':
> Provide the purpose of the trip, number of trips, number of days per trip, departure and arrival destinations, number of people, estimated rental car and airfare costs, and prevailing per diem rates as determined by gsa.gov, etc.; Quotes must be supported by screenshots from travel websites.
>
> 3) Regarding 'Equipment Purchases'
> Itemization with individual and total costs, including quantities, unit prices, proposed vendors (if known), and the basis of estimate (e.g., quotes, prior purchases, catalog price lists, etc.); any item that exceeds $5,000 must be supported with back-up documentation such as a copy of catalog price lists or quotes prior to purchase (NOTE: For equipment purchases, include a letter stating why the proposer cannot provide the requested resources from its own funding.

4) Regarding 'Materials'
Itemization with costs, including quantities, unit prices, proposed vendors (if known), and the basis of estimate (e.g., quotes, prior purchases, catalog price lists, etc.); any item that exceeds $5,000 must be supported with back-up documentation such as a copy of catalog price lists or quotes prior to purchase.

Best,

Luke Hamel
Program Assistant

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--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

PREEMPT budget (follow-up)

Luke Hamel <hamel@ecohealthalliance.org>

Fri 3/9/2018 6:23 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>

Hi Tonie,

As you are filling out your PREEMPT budget, there are a few additional comments I'd like to make:

- Regarding Section L, 'Budget Justification': Please begin drafting a **budget justification**, if you have not done so already. *This document must provide explanation for each line item within your detailed budget*. If you have any questions regarding this section, I'd be happy to provide you with additional information.
- Adding budget periods: If you refer to the budget template I sent to you earlier this week, you'll see that the top of page 2 reads, 'Research and Related Budget- Budget Period 1'.
    - This 'Budget Period 1' refers only to the first year of the proposed project. **In order to list expenses for subsequent years** (i.e. Year 2, Year 3, Option Year 1 and Option Year .5), you will have to **click the 'Add Period' button at the bottom of page 4** (just after Section L).
        - You will then see a page that is titled, 'Research & Related Budget- Budget Period 2', with accompanying sections (A-L) that are identical to those in Budget Period 1. Fill out this second budget period (copy over information from Budget Period 1 or edit as needed). You'll notice that the page titled, 'Research & Related Budget - Cumulative budget' updates as you add information to 'Budget Period 2.'
    - Repeat this process, adding periods for Year 3, Option Year 1 and Option Year .5
    - Attached to this email is a screenshot of the budget template document, highlighting where the 'Add Period' button can be found within the document.
- Budget 'Point of Contact': **Please let me know who is responsible for managing the PREEMPT budget at your institution**. We feel it will be easiest to communicate with this individual directly, to ensure nothing is lost when discussing budget-related items. If this individual is someone from another department at your institution, we will be sure to copy you on all emails.

Lastly, please return your budget (with all five 'budget periods' included) by Wed. 3/14, Eastern Time. We anticipate that you may have questions regarding certain aspects of the budget, so please do not hesitate to ask. We will try to resolve any issue as quickly as possible.

Best,

Luke Hamel
Program Assistant

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New York, NY 10001

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From: Luke Hamel <hamel@ecohealthalliance.org>
Sent: Friday, March 9, 2018 3:08 PM
To: Rocke, Tonie
Cc: Rachel Abbott; Aleksei Chmura; Dr. Peter Daszak; Alison Andre; Baric, Ralph S
Subject: Re: Rescheduling upcoming calls with Peter (PREEMPT)

Thank you, Tonie! Have an excellent weekend as well.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

[redacted] (direct)
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www.ecohealthalliance.org

On Fri, Mar 9, 2018 at 4:21 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hello Luke and Peter: Attached is my first draft of Task 7. Ralph Baric (copied here) and I had a good chat today about viral vectors and nanoparticles. We realized much of the delivery methods would depend on his work first, so there are some gaps here and the narrative will probably change after I see what Ralph has written. At any rate, this is something to at least start with. Have a good weekend! -Tonie
>
> On Thu, Mar 8, 2018 at 12:58 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > Once again**, please use this link, to select which date(s)/time(s) you are available to speak with Peter (select as many as you are available for)**. The goal is to have one call each week, on either Tuesday or Wednesday. Below, I've listed the dates/times that appear in the Doodle Poll link above. Please note that all times listed are in Eastern Time.
> >
> > Week 1:
> > Thu. 3/13 (9 AM - 5 PM ET)
> > Thu. 3/14 (9 AM - 5 PM ET)
> >
> > Week 2:
> > Thu. 3/20 (9 AM - 5 PM ET)
> > Thu. 3/21 (9 AM - 5 PM ET)
> >
> > Please let me know if you have any questions.
> >
> > Best,

Luke Hamel
Program Assistant

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--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

From: Rocke, Tonie <trocke@usgs.gov>
Sent: Friday, March 9, 2018 1:21 PM
To: Luke Hamel
Cc: Rachel Abbott; Aleksei Chmura; Dr. Peter Daszak; Alison Andre; Baric, Ralph S
Subject: Re: Rescheduling upcoming calls with Peter (PREEMPT)
Attachments: PREEMPT TR task 7 first draft.docx

Hello Luke and Peter: Attached is my first draft of Task 7. Ralph Baric (copied here) and I had a good chat today about viral vectors and nanoparticles. We realized much of the delivery methods would depend on his work first, so there are some gaps here and the narrative will probably change after I see what Ralph has written. At any rate, this is something to at least start with. Have a good weekend! -Tonie

On Thu, Mar 8, 2018 at 12:58 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Hi Tonie,
>
> Once again**, please use this link, to select which date(s)/time(s) you are available to speak with Peter (select as many as you are available for)**. The goal is to have one call each week, on either Tuesday or Wednesday. Below, I've listed the dates/times that appear in the Doodle Poll link above. Please note that all times listed are in Eastern Time.
>
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>
> Week 2:
> Thu. 3/20 (9 AM - 5 PM ET)
> Thu. 3/21 (9 AM - 5 PM ET)
>
> Please let me know if you have any questions.
>
> Best,
>
> **Luke Hamel**
> *Program Assistant*
>
> EcoHealth Alliance
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--
Tonie E. Rocke

USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

Task 7: Develop and assess delivery methods to bats for immune boosting and priming molecules

Description and execution: While work is proceeding to identify and optimize immunomodulating agents to manage SARS-Coronaviruses, we will concurrently develop and test mediums, routes, and methods of delivery to large colonies of bats. Several different approaches or combinations of approaches will be assessed to determine the most feasible and simplest method of delivery that achieves high uptake by bats, is safe for humans as well as target and non-target species, and minimizes disturbance to the colony. Sticky edible gels or pastes that bats groom from themselves and each other have been used previously to deliver pharmaceuticals to bats orally and are currently being tested as a medium for delivery of vaccines against rabies and other diseases in wild bats (see preliminary data). These may also be useful for delivering immune modulators and recombinant SARSr-CoV spike proteins to *Rhinolophus* bats, but may need to be combined with viral vectors (like poxvirus or adenovirus) or nanoparticles/nanoemulsions that enhance uptake through mucous membranes or transdermally after topical application.

Poxviruses in particular have been demonstrated to be effective viral vectors for delivering vaccines to wildlife (Slate et al., 2009) Freuling et al., 2013; Rocke et al., 2017). Recent laboratory studies in bats have shown that poxviruses can replicate safely at high levels in bats after oronasal administration (Stading et al., 2016)m and poxvirus vectored vaccines are immunogenic, protecting bats from rabies challenge (Stading et al 2017; see preliminary data). Poxviruses are highly safe, having been tested in a wide variety of wild and domestic animals, they allow for large inserts of foreign DNA, and they have a proven record of success. Poxviruses are good candidates for this project, but we will also consider others.

In addition to viral vectors, we will also consider methods to achieve transcutaneous delivery of the immune boosting proteins without the use of live agents. Recent advances in methods to achieve transdermal or transcutaneous delivery of drugs and vaccines have been reported. (Roberts et al., 2017). However, a major impediment to this route of vaccination is the stratum corneum, the outermost barrier layer of the skin that protects underlying layers from infection and damage. Numerous approaches have relied on mechanical methods to compromise the stratum corneum to allow the drug or vaccine to penetrate into the skin (Roberts et al., 2017). Innovations in nanotechnology show promise in being able to deliver drugs and vaccines into the deeper layers of the skin without the need for damage to the stratum corneum (Mishra et al., 2013), an important consideration. Dendritic cells and Langerhans cells, antigen-presenting cells which reside in the dermis and epidermis, can take up these transdermally delivered proteins and generate an immune response. We are currently testing poly lactic-co-glycolic acid (PLGA) as a nanoparticle to encapsulate rabies glycoprotein as a method of transcutaneous delivery of vaccine to bats. PLGA has been used previously to deliver both toll-like receptor agonists and antigens simultaneously to mice (Ebrahimian, 2017). This and other products (outlined above in Task ?) could potentially be useful with SARSr-CoV glycoproteins. Adjuvants can also be incorporated into nanoemulsions and nanoparticles to amplify the natural immune response to the vaccine antigens (Karande and Mitragotri, 2010). With SARS-CoV spike proteins, the adjuvant Matrix M1

(Isconova, Sweden) has been shown to significantly enhance the immune response in mice (Coleman et al. 2014)

In collaboration with Dr. Baric and others, we will determine the most likely immunomodulating formulations based on the results of TA2, previous animal studies and other available data and then use both laboratory and field studies to assess and optimize delivery vehicles and methods for wild bats. To reduce costs, initial studies will be conducted with locally acquired insectivorous bats (*Eptesicus fuscus*--big brown bats). We have successfully maintained and housed big brown bats and other insectivorous species for several experiments at our facility previously (Stading et al., 2016, 2017). We will treat bats via topical application with various test formulations that include the biomarker Rhodamine B (RB), co-house them with untreated bats, and monitor transfer between bats by collecting hair and whiskers for biomarker analysis. Rhodamine B is detectable within the hair of animals within 24 hours of consumption using a fluorescence microscope, and we have considerable experience using this biomarker for similar studies (see preliminary data).

Once we have confirmed uptake in laboratory studies, we will then assess mass delivery methods in local caves and hibernacula (using biomarker-labeled mediums but without immunomodulatory substances). We will test several different approaches including aerosolization via sprayers that could be used in cave settings and automated sprays triggered by timers and movement detectors at critical cave entry points. Within one week of application, bats will be trapped at the cave entrace using mist nets or Harp traps and hair will be collected to assess the rate of uptake via biomarker analysis. The bats will be released immediately afterward. The procedures will be tested at several different locations as it will likely take some manipulation to determine appropriate dosages for maximum uptake. After we have determined the most optimal approaches for mass delivery, we will then test them on wild bats in our three cave sites in Yunnan Province. Again, biomarker will be used to assess rates of uptake and this data can then be used in modeling studies to help determine the optimal rates of application of immunomodulating agents. Biomarker studies can also be used to assess uptake by non-target species, an important consideration in evaluating safety. Fieldwork will be conducted in collaboration with Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance).

Preliminary Data: Rocke and colleagues have developed oral vaccines and delivery methods to manage disease in free-ranging wildlife for many years, including a sylvatic plague vaccine for prairie dogs (Rocke et al., 2017), and more recently, vaccines against rabies (Stading et al., 2017) and white-nose syndrome for bats (Rocke, unpublished data). In addition to developing, testing and registering vaccines for experimental field use, vaccine delivery methods and uptake by the target species were optimized using biomarker studies prior to deployment; biomarker studies were also used to assess uptake and safety in non-target hosts (Tripp et al., 2015). A similar approach will be used to develop, test and optimize delivery methods to *Rhinolophus* bats in SE Asia.

To manage plague caused by *Yersinia pestis* in prairie dogs, a raccoon poxvirus vectored vaccine expressing plague antigens was incorporated into a peanut-butter flavored bait matrix. Rhodamine B (RB), a biomarker that dyes hair, whiskers and feces and is visible within 24 hours of consumption by animals, was included in the baits in

order to assess uptake by both target and non-target species (Figure 1). When viewed under a UV microscope at a specific wavelength, the biomarker is visible until the hair grows out (approximately 50 days in prairie dogs). Biomarker studies were initially used to assess palatability and acceptance of the bait matrix by wild prairie dogs (Tripp et al., 2014) and also used to assess bait ingestion by non-target rodents (Tripp et al., 2015). After safety was confirmed in non-targets and with the approval of USDA Center for Veterinary Biologics, a large field trial was conducted over a 3-year period that demonstrated vaccine effectiveness in four species of prairie dogs in seven western states (Rocke et al., 2017). Using biomarker analysis, we then assessed site- and individual host-level factors related to bait consumption in prairie dogs to determine those most related to increased bait consumption, including age, weight, and the availability of green vegetation. Identifying the factors that maximize the likelihood of expedient bait uptake by targeted individuals is important for developing strategies to optimize vaccine effectiveness. This will also be important in developing disease management strategies for bats.

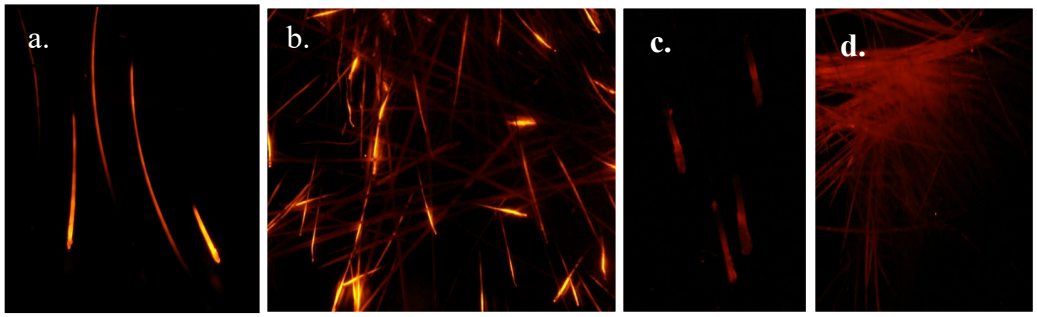

Figure 1. Prairie dog hair and whisker samples viewed under fluorescence microscope (excitation wavelength: 540 nm, emission wavelength: 625 nm) to determine uptake of baits containing Rhodamine B. a) whiskers positive for RB uptake 20 days after bait distribution, b) hair sample positive for RB uptake 16 days after bait distribution, c and d) whiskers and hair negative for RB uptake 20 days after bait distribution (note natural dull fluorescence).

In recent years, our research team has been developing and testing vaccines and delivery methods for use in free-ranging bats. First we tested two commonly used viral vectors, modified vaccinia Ankara (MVA) and raccoon poxvirus (RCN), for their safety and replication in bats using in vivo biophotonic imaging. (Stading et al. 2017). RCN replicated to higher levels in bats than MVA, even via the oral route, and was found to be highly safe for bats (Figure 2). We then used raccoon poxvirus as a viral vector to express a novel rabies glycoprotein (mosaic or MoG) and tested the protective efficacy of this construct in bats after both oronasal and topical administration (Stading et al 2017). Both methods of application were successful, protecting nearly all of the immunized and challenged bats (Figure 3), work is now progressing to develop methods of vaccine delivery to vampire bats, one of the primary reservoirs of rabies for both humans and animals, primarily cattle, in several Latin American countries. We are also using a similar approach to develop vaccines for white-nose syndrome in bats, a devastating disease that has killed millions of insectivorous bats in North America.

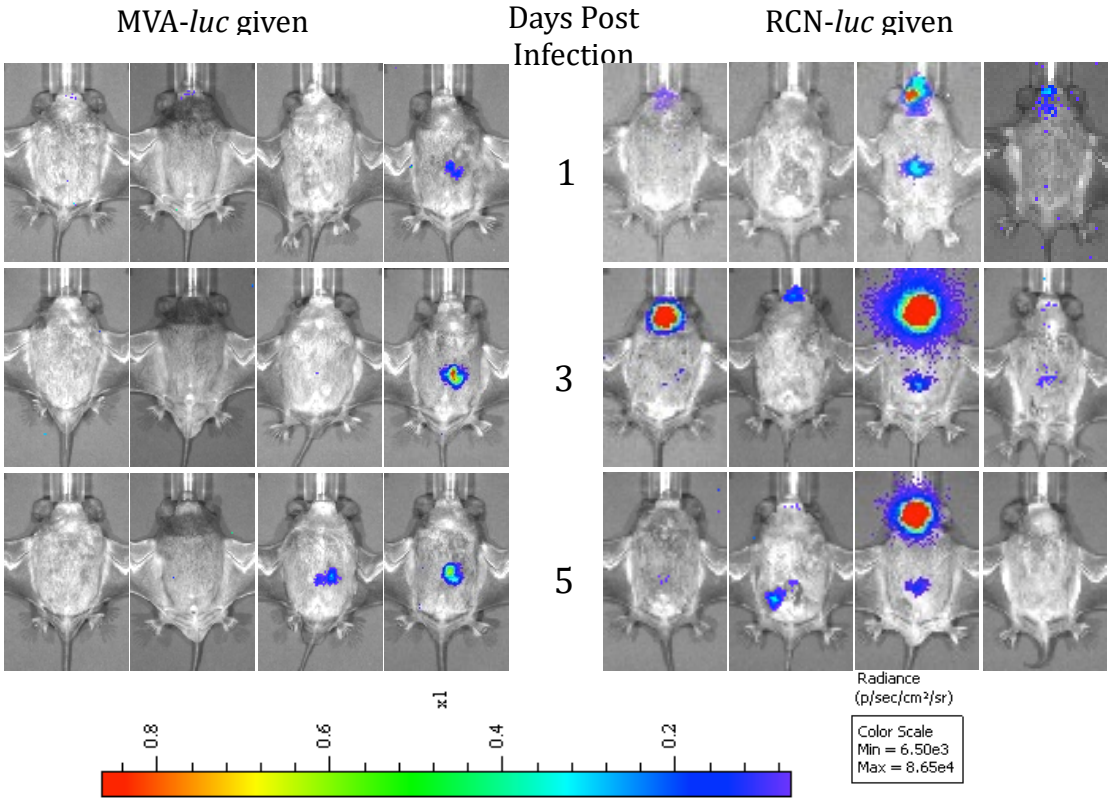

Figure 2. Luminescence, indicative of viral replication of modified vaccinia Ankara (MVA) and raccoon poxvirus RCN) in *Tadarida brasiliensis* on days 1, 3 and 5 post-inoculation via the oronasal route.

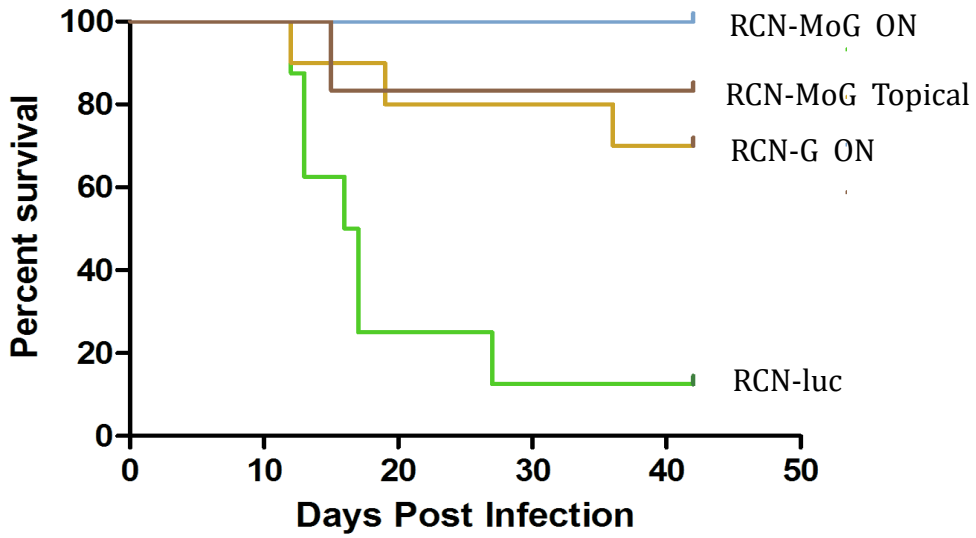

Figure 3. Results of vaccine efficacy and rabies challenge trials in *Epstesicus fuscus* immunized with raccoon poxvirus expressing a mosaic G protein (RCN-MoG) either oronasally (ON) or topically in comparison to RCN expressing typical G protein and RCN expressing luciferase (a negative control).

For bats a different approach is required for vaccine delivery, as in general, they are not attracted to baits. Bats, especially vampire bats, are known to practice self and mutual grooming at a high rate, and this behavior has been exploited to cull vampire bats using poisons like warfarin. The poison is applied topically to a number of bats that are released. When they return to their roost, the poison is transferred to roost-mates by contact and mutual grooming. We are exploiting this same behavior for vaccine application. Preliminary biomarker studies (without vaccine) are being conducted in vampire bats in both Mexico and Peru and also in insectivorous bats in Wisconsin. In a pilot study in Peru, we treated 50 bats from a single cave with RB-labelled glycerin jelly. Based on capture-recapture data, we estimated the population at ~200 bats, so ~25% of bats were initially marked. Upon trapping of this population a few days later, 64 bats were captured, including 19 originally marked bats (Table 1 – could be made into a figure instead). Hair was collected and examined for RB marking under a fluorescence microscope. All treated bats were positive for RB marking in addition to 39% of newly captured bats, indicating a rate of transfer of about 1.3 bats for every bat marked. Additional trials have been conducted, with transfer rates of up to 2.8 bats for every bat treated achieved at least once. These trials are being analyzed to assess factors associated with rates of transfer, e.g. sex and age of initially treated bats, time of day, etc. This data is then being used to model the rate of vaccination and impact on rabies transmission with different rates of application, prior to actual deployment of vaccine in the field.

Table 1. Marking of vampire bats a few days after application of glycerin jelly containing Rhodamine B.

	Number captured	**Positive**	**Negative**	**Inconclusive**	**% positive (w/o inc)**
All bats	64	34	25	5	58
Recaptured marked bats	19	18	0	1	100
New bat captures	45	16	25	4	39

For insectivorous bats, we are trying other approaches. Instead of hand applying the jelly to bats, we applied RB marked glycerin jelly to the entry of bat houses used by little brown bats (*Myotis lucifugus*). The bats became covered as they entered the houses and then consumed the material during self and mutual grooming. One week later, bats were trapped at the houses to determine the rate of uptake. Of 29 bats trapped one week post-application, 59% (17) were positive for biomarker indicating they had eaten the jelly. Thus, with additional optimization, application of vaccine to bat houses or other

structures (small cave entrances) could also be a viable method of delivery. In addition, we are considering different spray applications directly to roosting bats in caves and through motion-sensing sprayers at cave entrances. Whatever the means of application, effective treatment relies on ingestion by bats, and that is easily confirmed with the use of the biomarker, RB.

Organization leading task: USGS National Wildlife Health Center

Progress Metrics: Not sure exactly what format to use here

Deliverable(s):
Medium and methods to deliver immunomodulatory agents to bats.
Data on uptake in insectivorous bats.
Reports, manuscripts, presentations.

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Karande P, Mitragotri S. 2010. Transcutaneous immunization: an overview of advantages, disease targets, vaccines, and delivery technologies. Annu Rev Chem Biomol Eng 1:175-201.

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Rocke TE, Tripp DW, Russell RE, Abbott RC, Richgels KLD, Matchett MR, Biggins DE, Griebel R, Schroeder G, Grassel SM, Pipkin DR, Cordova J, Kavalunas A, Maxfield B, Boulerice J, Miller MW. 2017. Sylvatic plague vaccine partially protects prairie dogs (*Cynomys* spp.) in field trials. EcoHealth DOI: 10.1007/s10393-017-1253-x.

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Tripp DW, Rocke TE, Streich SP, Brown NL, Fernandez JR-R, Miller MW. 2014. Season and application rates affect vaccine bait consumption by prairie dogs in Colorado and Utah, USA. J Wildlife Dis 20:

Tripp DW, Rocke TE, Streich SP, Abbott RC, Osorio JE, Miller MW. 2015. Apparent field safety of a raccoon poxvirus-vectored plague vaccine in free-ranging prairie dogs, Colorado, USA. J Wildlife Dis 51:

RE: Rescheduling upcoming calls with Peter (PREEMPT)

Baric, Ralph S <rbaric@email.unc.edu>

Sun 3/11/2018 7:52 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Its rough, but here's a draft. One section not included yet. Ralph

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Friday, March 9, 2018 4:21 PM
To: Luke Hamel <hamel@ecohealthalliance.org>
Cc: Rachel Abbott <rabbott@usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Dr. Peter Daszak <daszak@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>
Subject: Re: Rescheduling upcoming calls with Peter (PREEMPT)

Hello Luke and Peter: Attached is my first draft of Task 7. Ralph Baric (copied here) and I had a good chat today about viral vectors and nanoparticles. We realized much of the delivery methods would depend on his work first, so there are some gaps here and the narrative will probably change after I see what Ralph has written. At any rate, this is something to at least start with. Have a good weekend! - Tonie

On Thu, Mar 8, 2018 at 12:58 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Hi Tonie,
>
> Once again**, please use this link, to select which date(s)/time(s) you are available to speak with Peter (select as many as you are available for)**. The goal is to have one call each week, on either Tuesday or Wednesday. Below, I've listed the dates/times that appear in the Doodle Poll link above. Please note that all times listed are in Eastern Time.
>
> Week 1:
> Thu. 3/13 (9 AM - 5 PM ET)
> Thu. 3/14 (9 AM - 5 PM ET)
>
> Week 2:
> Thu. 3/20 (9 AM - 5 PM ET)
> Thu. 3/21 (9 AM - 5 PM ET)
>
> Please let me know if you have any questions.
>
> Best,
>
> **Luke Hamel**
> *Program Assistant*
>
> EcoHealth Alliance
> 460 West 34th Street – 17th floor
> New York, NY 10001
>
> (b) (6) (direct)
> (b) (6) (mobile)
> www.ecohealthalliance.org

> ***EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.***

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
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trocke@usgs.gov

***(TA1)* Task 4: Develop recombinant chimeric spike proteins from characterized SARSr-CoVs**

***TA1.a. Description and execution**:* Our international team's 15 yrs work experience on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014, WIV-1, WIV-16). The pre-epidemic potential of these SARSr-CoVs is high, as our groups have shown these SARS-like bat viruses use human, bat and civet angiotensin-1 converting enzyme 2 receptors (ACE2) for entry, and importantly, bind and replicate efficiently in primary human lung airway cells, like epidemic SARS-CoV (PMC4801244, PMC4797993). Moreover, chimeras (recombinants) with SARSr-CoV spike proteins in a SARS-CoV backbone, as well as synthetically reconstructed full length SCHO14 and WIV-1 SARS-like bat viruses cause SARS-like illness in humanized mice (express human ACE2 receptor), with clinical signs that are not reduced by SARS monoclonal therapy or vaccination (PMC4801244, PMC4797993). We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV (*7,8,9*). We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort) (PMID:29500691), suggesting active spillover, and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**.

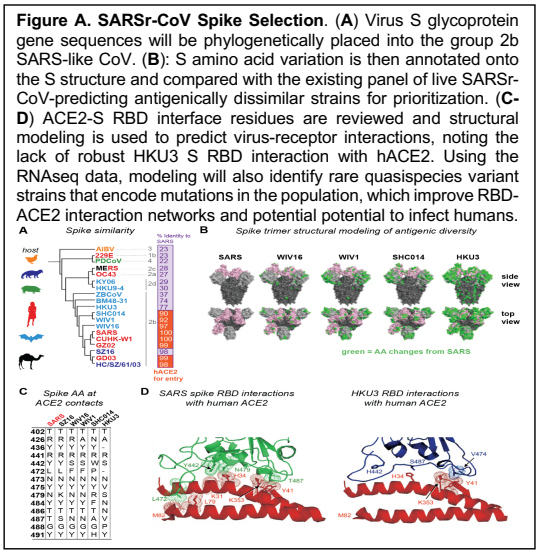


Figure A. SARSr-CoV Spike Selection. (**A**) Virus S glycoprotein gene sequences will be phylogenetically placed into the group 2b SARS-like CoV. (**B**): S amino acid variation is then annotated onto the S structure and compared with the existing panel of live SARSr-CoV-predicting antigenically dissimilar strains for prioritization. (**C-D**) ACE2-S RBD interface residues are reviewed and structural modeling is used to predict virus-receptor interactions, noting the lack of robust HKU3 S RBD interaction with hACE2. Using the RNAseq data, modeling will also identify rare quasispecies variant strains that encode mutations in the population, which improve RBD-ACE2 interaction networks and potential potential to infect humans.

TA1.b. SARSr-CoV Sample Collection and CoV Species Specificity. The Wuhan Institute of Virology team will continue to collect biodiversity surveys from SARSr-CoV viruses of bat caves across S. China. They have large, but incomplete collections of SARSr-CoVs sequences, most of which have not been evaluated for pre-epidemic potential. SARS-CoV cross species transmission is heavily regulated by S glycoprotein receptor binding domain interaction with the angiotensin 1 converting enzyme 2 receptor (ACE2). The structure of the SARS trimer prefusion spike has been solved as well as the bound SARS S RBD-ACE2 complex (PMC5223232, PMC4860016,) Mutations in the RBD regulate SARSr-CoV capacity to replicate in human, civet, mouse and bat cell lines and animals (PMC2588415, PMC2258931, PMID:16166518, PMC2446986). In fact, human-optimized RBD residues (Phe-442, Phe-472, Asn-479, Asp-480, and Thr-487) bind hACE2 best while civet-optimized RBD (Tyr-442, Pro-472, Arg-479, Gly-480, and Thr-487) bind cACE2 best but also hACE2 (PMC3308800). In addition, recent data demonstrate that host proteases and S glycoprotein proteolytic processing also regulates entry and cross species transmission efficiency (PMID:16339146, PMC4151778, PMC5552337). If mismatches occur in the S-RBD-ACE2 molecules or in S proteoloytic processing, SARSr-CoV will not enter and hence cannot replicate in cells, unless this RBD-ACE2 interface is repaired by reverse genetics or mutation (PMC2588415, PMC2258931). Importantly, CoV species specific restriction events are limited after entry, as this same viral genome is replication competent when delivered by transfection or electroporation (PMC2588415, PMC2258931).Consequently, entry functions represent the key first step to evaluating the disease potential of SARSr-CoV.

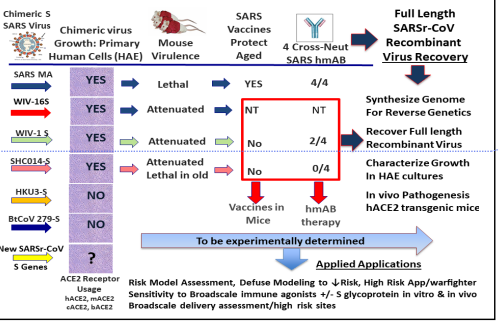
Using RNAseq and Sanger sequencing methods, our groups have collected full length genome and S glycoprotein gene sequences over time and analyzed S genes analyzed phylogenetically for recombination events, and high-risk viruses by our group and others in the program (e.g., RBD-ACE2 structure modeling, receptor binding domain (RBD) sequence conservation, spike glycoprotein similarity to SARS-CoV, and protease cleavage site bioavailability, etc.). From in silico data and risk assessment modeling platforms, the primary goals in Task4 are to: **i**) model and down-select strains for recovery and analyses, **ii**) identify S glycoprotein genes which program infection in vitro using pseudotyped and chimeric viruses, iv) synthetically reconstruct a panel of high risk full length SARSr-CoV strains, **v**) characterize virus growth phenotypes in primary human airway cultures and vi) perform in vivo pathogenesis studies using human ACE2 transgenic mice.

Commented [BRS1]: Need linkage to modeling group.

TA1.c. Preliminary Data: The Baric laboratory pioneered many of the strategic approaches (i-vi) and the SARSr-CoV reverse genetic platforms used for coronaviruses, including the use of synthetic genome design to reconstruct and recovery full length or S chimeric recombinant viruses from in silico sequence database (PMC4801244, PMC4797993, PMC2588415, PMC2258931). An example of full length recombinant SARSr-CoV reconstructed using reverse genetics in the Baric laboratory is shown in **Fig A, Panel B** and include human epidemic strains, civet and raccoon dog SARS-CoV strains, as well as SARSr-CoV strains (WIV16, WIV1, SHC014 and HKU3-SRBD {repaired RBD interface)}. These strains are immediately available for use in assessment of broadscale approaches to reduce population burdens of SARSr-CoV in bat cells available in the Baric, Shi and Wang laboratories (PMC2258931, PMC4801244, PMC4797993, PMC2588415, PMC4801244, PMC4797993).

TA1.d. Novel SARSr-CoV Virus Recovery. *Chimeric Viruses*. Our approach for testing the pre-epidemic potential of novel SARS-rCoV strains identified by sequence analyses is shown in **Figure B**. First, we will commercially synthesize selected SARSr-CoV S glycoprotein genes, designed for direct insertion into our full length SCH014 or WIV16 molecular clones (BSL3, not select agent, pathogenic in hACE2 transgenic mice), noting that these two SARSr-CoV are 88 and 97% identical to epidemic SARS-Urbani in the S glycoprotein. As the S gene interacts with the M glycoprotein, E protein and N protein in assembly/release, different backbone strains provide increased opportunities for recovery of viable viruses, as well as to identify potential barriers for RNA recombination between strains (PMC5708621). The chimeric viruses will be recovered in Vero cells, or in mouse cells over-expressing human, bat and civet ACE2 receptors, as receptor over-expression may support cultivation of viruses having weak RBD-ACE2 interfaces and sequence verified. Viruses will then evaluated for: **i**) human, civet and bat ACE2 receptor usage in vitro, **ii**) growth in primary human airway epithelial cells, **iii**) sensitivity to broadly cross neutralizing human monoclonal antibodies S215.17, S109.8, S227.14 and S230.15 and a mouse antibody (435) that recognize unique epitopes in the RBD (PMC2826557, PMID:29134417) and **iv**) in vivo pathogenesis studies in hACE2 transgenic mice, using well established approaches in our laboratory (). A limited number of human SARS serum samples from the Toronto outbreak in 2003 are also available to evaluate cross neutralizing profiles using polyclonal serum (n=10), should some isolates prove highly resistant to our panel of mAB. Chimeric viruses that encode novel S genes with pre-epidemic potential (e.g., growth in HAE, use of multiple species ACE2 receptor for entry, antigenic variation, etc.) will be used to identify SARSr-CoV strains for recovery as full genome length viable viruses. We anticipate testing ~20 S genes/yr.

Figure B. Experimental Design. S genes of interest will be inserted into WIV16 and/or SCH014 full length molecular clones and chimeric virus rowth evaluated in primary human cells and cell lines constitutively expressing bat, civet, human and mouse ACE2 receptors. In vivo pathogenesis, hmAB cross neutralization assays and therapeutic intervention studies in vivo will prioritize strains for recovery of full-length SARSr-CoV.



TA1e. Recovery of Full length SARSr-CoV. To recover full length viruses, we will first compile the sequence/RNAseq data from a panel of closely related strains (e.g,<5% nucleotide variation) and compare the full length genome sequences, scanning for unique SNPs which might represent sequenceing errors as previously described by our groups (PMC3497669, PMC2583659, PMC3791741). We will identify the best consensus candidate and synthesize the genome using commercial vendors (e.g., BioBasic, etc.), as six contiguous cDNA pieces linked by unique restriction endonuclease sites that do not disturb the coding sequence, but allow for full length genome assembly. Full length genomes will be transcribed into RNA and electoration is used to recovery full length recombinant viruses (PMC3977350, PMC240733). Using the full length genomes, we will re-evaluate virus growth in primary human airway epithelial cells at low and high multiplicity of infections and in vivo in hACE2 transgenic mice, testing whether backbone genome sequence alters full length SARSr-CoV pre-epidemic or pathogenic potential in models of human infection. All experiments are performed in triplicate and the data provided to the Modeling Team for the development of risk assessment models, warfighter apps, and models to evaluate potential intervention outcomes. We anticipate recovering 2-5 full length genomes/yr, reflecting strain differences in antigenicity, receptor usage, growth in human cells and pathogenesis.

Commented [BRS2]: Modeling team, how many you need; 60+ provide much help for modeling. I note that if we validate 1, half-a dozen additional strains with slight sequence variation also will fall in the "pre-epidemic" high risk categories so multiplies will get you into the 100's or 1000's of strains.

TA1.f. In vivo Pathogenesis Studies. To generate a mouse model more relevant to humans, we generated a mouse that expresses human ACE2 receptor under control of HFH4, a lung ciliated epithelial cell promoter (PMC4801244). Infection of this model with wildtype SARS-CoV and WIV1 resulted in lethal disease outcomes with SARS-CoV, but minimal disease with WIV1. These data argue that WIV1 is less likely efficient at using the hACE2 receptor in vivo and hence less likely to produce severe disease outcomes in an outbreak setting. Of note, microvariation in the SARs-CoV RBD of related strains could dramatically alter these phenotypes, hence the need to evaluate the impact of low abundant, high consequence microvaration in the RBD. Briefly, groups of 10 animals will be infected intranasally with 1.0 x 104 PFU of each virus. Clinical disease (e.g., weight loss, respiratory function by Buxco plethysmography, mortality) will be followed for 6 days. One half the animals will be sacrificed at day 2 and 6 postnfection for virologic determinations, histopathology and immunohistochemistry in the lung and for 22-parameter complete blood count (CBC) in blood and BAL using the Vetscan HM5.

TA1.g. Evaluating 2^{ary} S gene Markers for SARSr-CoV Pre-epidemic Potential. 1) ***Identification of high risk/low abundant variants***. RNAseq will identify low abundant quasispecies variants that encode mutations in the RBD and/or residues that bind ACE2 receptor (Fig A). Low abundant mutations, especially in RBD residues that interface with ACE2 receptors, would alter risk assessment calculations as strains identified as low risk, might actually encode high risk, but low abundant variants. To test this hypothesis, we will closely with the modeling core and Dr. Shi's laboratory to identify highly variable residue changes in the SARSr-CoV S RBD, and use commercial gene blocks to introduce these changes singly and then in combination into the S glycoprotein gene of the parental low risk, high abundant strain parent. We will evaluate the ability of these low abundant chimeric viruses to use human, bat, civet and mouse ACE2 receptors, and more importantly, replicate efficiently in human primary cells. 2) ***Impact of RBD deletions on Pre-epidemic Risk Assessment***. SARSr-CoV RBD sequences fall into two larger clades, heavily defined by the presence of small deletions between residues 432-437 and 458-472, which leave the key RBD-ACE2 interface residues intact. (**Fig C**). To improve risk assessment, we will molecularly analyze the functional consequences of these deletions on SARSr-CoV human ACE2 receptor usage, growth in primary cells and in vivo pathogenesis. First, we will delete these regions, sequentially and then in combination, in SCH014, anticipating that the introduction of both deletions will prevent SCH014 growth in Vero and human cells. We also hypothesize that the smaller deletion may be tolerated, given it location in the RBD structure. In parallel, we will evaluate the ability of targeted recombination between high and low risk strains to restore pre-epidemic features to low risk viruses. To test this hypothesis, we will synthesize full length rs4237, a highly variable SARSr-CoV that encodes the SHC014 RBD contact interface residues at 442, 487 and 491 but also encodes mutation at 479 (N479S) and has the 432-437 and 458-472 deletions and hence, is not recoverable in vitro. Using the SCH014 backbone sequence, we will first sequentially and then in tandem repair the 432-437, 458-472 deletions in the presence and absence of the S479N. We anticipate that the S479N mutation is critical given its key role in establishing the RBD-ACE2 interface, and that restoration of the RBD deletions will significantly enhance virus recognition of hACE2 receptors and growth in Vero and HAE cultures in vitro. Together, these data will directly inform risk assessment SARSr-CoV population genetic structure obtained from difference cave ecologies. **3) *S2 Proteolytic Cleave and Glycosylation Sites***. In some instances, recombinant chimeric viruses may be predicted to replicate efficiently because of matched RBD-ACE2 interfaces, yet fail to replicate. After receptor binding, cell surface or endosomal proteases cleave the SARS S glycoprotein to activation fusion mediated entry (PMC4151778). Massive changes in Spike structure occur to mediate membrane fusion and entry (PMC5651768). The absence of S cleavage prevents SARS-coV entry (PMC5457962). A variety of proteases, including TMPRSS2, TMPRSS11a, HAT, trypsin and cathepsin L carry out these processes on the SARS S glycoprotein (*PMC3233180, PMC3889862, PMC5479546, PMID:26206723*)(**Fig C**). In some instances, tissue culture adaptations introduce a furin cleavage site, which can direct entry processes as well, usually by cleaving S at positions 757 and 900 in S2 of other coronaviruses, but not SARS (*PMID:26206723*). For SARS-CoV, a variety key cleavage sites in S have been identified including R667/S668, R678/M679 for trypsin and cathepsin L, respectively, R667 and R792 (and other unidentified sites) for TMPRSS2, and R667 for HAT. Therefore, all

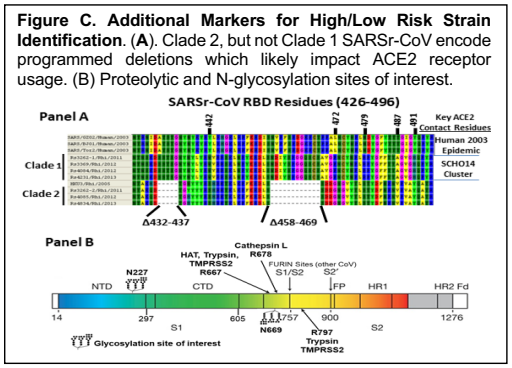


Figure C. Additional Markers for High/Low Risk Strain Identification. (**A**). Clade 2, but not Clade 1 SARSr-CoV encode programmed deletions which likely impact ACE2 receptor usage. (B) Proteolytic and N-glycosylation sites of interest.

SARSr-CoV S gene sequences will be analyzed for the presence of these appropriately conserved proteolytic cleavage sites in S2 and for the presence of potential furin cleavage sites (R-X-[K/R]-R↓) and which can be predicted computationally (PMC3281273) . Importantly, SARr-CoV with mismatches in proteolytic cleavage sites can be activated by exogenous trypsin or cathepsin L (Fig D), providing another strategy to recover non-cultivatable viruses. In instances where clear mismatches occur in these S2 proteolytic cleavage sites of SARSr-CoV, we will introduce the appropriate human-specific cleavage sites and evaluate growth potential in Vero and HAE cultures. In SARS-CoV, we will ablate several of these sites based on pseudotype particle studies and evaluate the impact of select SARSr-CoV changes on virus replication and pathogenesis (e.g., R667, R678, R797). Experimental outcomes from these studies will be incorporated into risk management models to identify high and low risk SARS-like CoV.

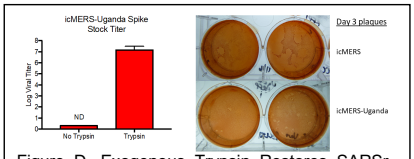


Figure D. Exogenous Trypsin Restores SARSr-CoV Growth.

SARS S has 23 potential N-linked glycosylation sites N-linked glycosylation sites (NX(S/T; X is anything but proline) and 13 of these have been confirmed using biochemical approaches (e.g., positions: 118, 119, 227, 269, 318, 330, 357, 783, 1056, 1080, 1140, 1155, and 1176). Importantly, several N-linked glycosylation sites regulate SARS particle binding DC-SIGN/L-SIGN, alternative entry receptors for SARS-CoV infections (positions: N109, N118, N119, and especially N227, N699)(PMC1641789, PMC2168787, PMC2168787) and may protect critical sites for antibody neutralization(PMC5515730). Importantly, the emergence of human SARS-CoV from civet and raccoon dog reservoir strains was associated with the evolution of mutations that introduced two N-linked glycosylation sites that promote DC-SIGN/L-SIGN binding (N227, N699), suggesting a role in the expanding human 2003 epidemic (PMC2168787). Interesting, these N-linked glycosylation sites are absent from civet, raccoon dog strains and clade 2 SARSr-CoV, but are present in WIV1, WIV16 and SCH014 as well as human epidemic strains, supporting a potential role in host jumping (**Fig C**). To evaluate the role of these mutations in cross species transmission and pathogenesis, we will sequentially introduce clade 2 residues at positions N227 and N699 of SARS-CoV and SCH014 and evaluate virus growth in Vero, Huh7 cells (nonpermissive) expressing ectopically expressed DC-SIGN and HAE cultures, anticipating reduced virus growth efficiency. Using the clade 2 rs4237 molecular clone described above, we will introduce the clade I mutations that introduce N-linked glycosylation sites at positons 227 and N699 and in rs4237 RBD deletion repaired strains, evaluating virus growth efficiency on Vero, HAE or Hela cells ± ectopically expressing DC-Sign (PMC2168787). In vivo, we will evaluate pathogenesis in transgenic ACE2 mice. Experimental outcomes from these studies will be incorporated into risk management models to identify high and low risk SARS-like CoV.

Organization leading task: University of North Carolina

Progress Metrics: Not sure how to do this.

Deliverable(s):

1. *Methods to Produce Synthetic SARSr-CoV Virus Molecular Clones and Reverse Genetics.*
    a. ***Preliminary Data**:* Molecular Clones for SARSr-CoV WIV1, WIV16, SCH014 and HKU3-SRBD exist. We have demonstrated in the preliminary data that these reagents are already available.
    b. ***Target Goals**:* We will generate molecular constructs for 20+ chimeric SARSr-CoV encoding different S glycoprotein genes/yr
    c. **Target Goals***: We will generate 2-5 full length molecular clones of SARSr-CoV.*
2. *Methods of Recombinant virus Recovery and Characterization*
    a. **Preliminary Data***: Demonstrated recovery recombinant chimeric SARSr-CoV WIV1, WIV16, SCH014, HKU3-SRBD, including full length recombinant viruses of WIV1, WIV16, SCH014 and HKU3-SRBD.*
    b. *Target Goals:* We will isolate 20+ chimeric SARSr-CoV encoding novel S glycoprotein genes
    c. *Target Goals:* We will isolate 2-5 full length SARSr-CoV/year/
        i. **Key Deliverables for Program-wide Success***: These two key reagents position us for immediate testing of the antiviral effects of* **broadscale immune boosting molecules** +/- immunogens on virus growth in vitro and in vivo, and on virus levels in models of

chronic SARS-CoV infection in mice.

3. *Virus Phenotyping: Receptor Interactions and In Vitro Growth.*
    a. ***Preliminary Data**: Cell lines encoding bat, human, civet and mouse ACE2 receptors exist and have been validated. We have demonstrated the use of primary human airway epithelial cultures to characterize SARSr-CoV pre-epidemic potential.*
    b. ***Target Goals**: We will characterize SARSr-CoV recombinant virus growth in Vero cells, nonpermissive cells encoding the civet, bat and human ACE2 receptors.*
4. *Virus Pathogenic Potential in Humans:*
    a. *Preliminary Data: We also have transgenic human ACE2 mouse models to compare the pathogenic potential of SARSr-CoV*
    b. *Target Goals: We will evaluate SARSr-CoV pathogenic outcomes in hACE2 transgenic mice.*
5. *Virus Antigenic Variation:*
    a. *Preliminary Data: We have robust panels of broadly cross reactive human monoclonal antibodies against SARS and related viruses and mouse models to evaluate protection against SARSr-CoV replication and pathogenesis.*
    b. *We will evaluate SARS-vaccine performance against a select subset of SARSr-CoV (10), chosen based on the overall percent of antigenic variation, coupled with distribution across the S glycoprotein structure.*
6. *Low Abundant High Consequence Sequence Variants:*
    a. *We will identify the presence of low abundant, high risk SARSr-CoV, based on deep sequencing data*
7. *Proteolytic Processing and Pre-epidemic Potential.*
    a. *We will evaluate the role of proteolytic cleavage site variation on SARSr-CoV cross species transmission and pathogenesis in vivo.*

(TA2) **Task 6: Trial experimental approaches aimed towards 'Immune Targeting' using experimental bat colonies**

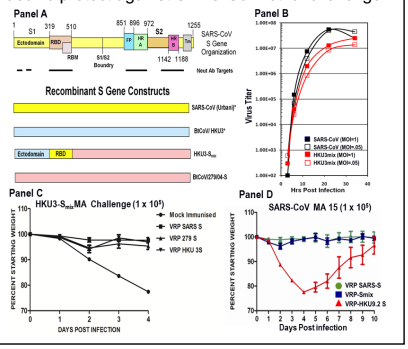
***TA2.a. Description and execution**:* There is no available current technology to reduce the risk of exposure to novel coronaviruses from bats which carry zoonotic precursors to many emerging viruses including filoviruses (Ebola), Coronaviruses (SARS-CoV, MERS-CoV, etc.), paramyxoviruses (Nipha/Hendra), rhabdoviruses (rabies) and others. Unfortunately, models of bat host capacity to harbor viruses, of ecological and environmental drivers of their emergence, and of the evolutionary potential of different strains to spillover are rudimentary. No vaccines or therapeutics exist for emerging coronaviruses, filoviruses and paramyxoviruses and exposure mitigation strategies are non-existent. Using emerging coronaviruses (SARSr-CoV) as models, we will apply **broadscale immune boosting strategies** alone (Prof. Linfa Wang (Duke-NUS) or in the presence and absence of chimeric immunogens (**targeted immune boosting strategy**), designed to upregulate bat immunity in the cave roosts, down-regulate viral replication and boost adaptive immunity to high risk strains. We will use small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists coupled with novel chimeric polyvalent recombinant spike proteins in microparticle encapsidated gels and powders for oral delivery and/or virus adjuvanted immune boosting strategies where chimeric recombinant SARSr-CoV spikes are expressed from raccoon poxvirus, which has been used extensively to devlier rabies immunogens in bats and other animals. We will design novel methods to deliver these applications remotely to reduce exposure risk during decontamination.

Commented [BRS3]: Broadscale immune boost + chimeric immunogen

The Baric group has developed novel group 2b SARSr-CoV chimeric S glycoproteins that encode neutralizing domains from phylogenetically distant strains (e.g., Urbani, HKU3, BtCoV 279), which differ by ~25%. The chimeric spike programs efficient expression when introduced in the HKU3 backbone full length genome, and elicit protective immunity against multiple group 2b strains (see preliminary data). We will use this platform as a broadscale immune boosting strategy. First, we will develop robust expression systems to express SARSr-CoV chimeric spikes using ectopic expression in vitro. Then, we work with Dr. Ainslie (UNC-Pharmacy) who has developed novel microparticle delivery systems and dry

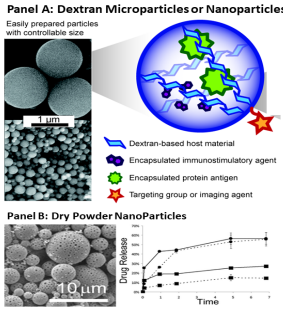
powders for aerosol release, and which encapsidate recombinant proteins and adjuvants (innate immune agonists) that will be used for parrellel **broadscale immune boosting strategies ± chimeric immungens.** In parallel, we will introduce chimeric and wildtype spikes in raccoon poxvirus (RCN), in collaboration with Dr. Rocke and confirm recombinant protein expression, first in vitro and then in bats in collaboration with Dr. Shi and Dr. Wang, who have bat colonies. The goal of this aim is to develop a suite of reagents to remotely reduce exposure risk in high risk environmental settings.

Figure E. Chimeric SARSr-CoV S Glycoprotein Immunogens. (A) A chimeric S glycoprotein was synthesized which contained HKU3, SARS-CoV and BtCoV/279/04. (B) Recombinant viruses encoding the HKU3-Smix gene were viable and grew to ~108 PFU/ml in Vero Cells. (C) VRP vaccines encoding the SARS-S, BtCoV 279-S and HKU3-S protect against HKU3-Smix challenge. (D) VRP-HKU3-Smix vaccine protect against SARS-CoV lethal challenge.



TA2.b. Preliminary Data: Chimeric SARSr-CoV Spike Immunogens. Coronaviruses evolve quickly by mutation and RNA recombination, the latter provides a strategy to rapidly exchange functional motifs within the S glycoprotein and generate viruses with novel properties in terms of host range and pathogenesis (PMC237188, PMC5708621). Coronaviruses also encode neutralizing epitopes in the NTD, RBD and S2 portion of the S glycoprotein (PMID:29514901, PMC2826557, PMC2268459, PMC3256278). Given the breadth of SARSr-CoV circulating in natural settings, chimeric immunogens were designed to increase the breadth of neutralizing epitopes across the group 2b phylogenetic subgroup (PMC5708621). Using synthetic genome design and structure guided design, we fused the n-terminal NTD domain of HKU3 (1-319) with the SARS-CoV RBD (320-510) with the remaining BtCoV 279/04 S glycoprotein molecule (511-1255), introduced the chimeric S glycoprotein gene into the HKU3 genome backbone (25% different than SARS-CoV, clade 2 virus) and recovered viable viruses (HKU3-S_{mix}) that could replicate to titers of about 10^8 PFU/ml on Vero cells (Fig E). HKU3-Smix is fully neutralized by monoclonal antibodies that specifically target the SARS RBD (data not shown). In parallel, we inserted the $HKU3_{mix}$ S glycoprotein gene into Venezuelan equine encephalitis virus replicon vectors (VRP-$S_{chimera}$) and demonstrated that VRP vaccines protect against wildtype SARS-CoV challenge and virus growth. In addition, VRP-SHKU3 and VRP-S279 both protect against HKU3mix challenge and growth in vivo (Fig E), demonstrating that neutralizing epitopes in the $HKU3_{mix}$ S glycoprotein are appropriately presented and provide broad cross protection against multiple SARSr-CoV. In addition to using these immuogens as a targeted broad-based boosting strategy in bats, we will also produce a chimeric SCH014/SARS-CoV/HKU3 S gene for more focused immune targeting on known high risk strains.

Figure F. Particle Delivery Systems. Broadscale immune boosting strategies include (A) Dextran microparticles or (B) Dry Powders.



In parallel, we will work with the Protein Expression Core at UNC (https://www.med.unc.edu/csb/pep)to produce codon optimized, stabilized and purified prefusion SARS-CoV glycoprotein ectodomains as described by Pallesen J et al., PNAS 2016. Briefly, the chimeric S ectodomain will be linked to a C-terminal T4 fibritin trimerization domain, an HRV3c cleavage site, an 8xHis-Tag and a Twin-Strep-tag, and after transfection, mg quantities will be produced in 0.5–1 L FreeStyle 293-F cells treated with kifunensine (5 µM final concentration) for 6 d. The chimeric S trimer protein will be purified Strep-Tactin resin (IBA), treated withHRV3C protease overnight at 4 °C and the products purified using a Superose 6 16/70 column (GE Healthcare Biosciences) (PMC5584442). Purified recombinant protein will be used by Dr. Rocke and Dr. Ainslie for inclusion in delivery matrices (e.g., purified powders, dextran beads, gels) with broadscale immune agonists (adjuvants-Dr. Wang) like poly IC, TLR4 and Sting agonists and can be fine-tuned for regulated delivery by programmed degradation for time-ordered delivery (Fig F). These particles can be aerosolized, or delivered in sprays or gels to bat populations, providing new modalities for zoonotic virus disease control in wildlife populations (PMID: 28032507, PMC4267924).

TA2.c. 2nd Generation Chimeric S glycoprotein Design and Testing. Using the approach discussed above, we will also produce a chimeric SCH014 NTD/SARS-CoV-RBD/HKU3 S C terminal and generate recombinant HKU3 encoding the

trimer spike (HKU3-S_{S014}), for more focused immune targeting on known high risk strains with pre-epidemic potential. After sequence variation, we will evaluate virus growth in Vero and HAE cultures and the ability of SARS RBD monoclonal antibodies (S227, S230, S109) to neutralize chimeric virus infectivity (PMC2268459, PMC2826557). We will also evaluate in vivo pathogenesis in C57BL/6 mice and hACE2 transgenic mice. The recombinant HKU3-S_{S014} S genes will be introduced into VRP vectors and sent to Dr. Rocke for insertion into the raccoon poxvirus vaccine vector, characterization of S expression and then provided to Drs. Wang and Shi for immune boosting of bats. Recombinant HKU3-SS014 glycoprotein expression will be validated by Western blot and by vaccination of mice, allowing us to determine if the recombinant protein elicits neutralizing antibodies that protect against lethal SARS-CoV, HKU3-S_{mix} and SCH014 challenge. In parallel, we will survey the RNAseq data for evidence of complex S glycoprotein gene RNA recombinants in the cave SARSr-CoV population genetic structure. We will synthesize 2-3 interesting recombinant S genes, insert these genes into SCHO14 or HKU3 genome backbones and VRP and characterize the viability and replicative properties of these viruses in cell culture and in mice and the VRP for S glycoprotein expression and vaccine outcomes.

TA2.d. Microparticle Performance Metrics in vitro and in Rodents and Bats.

Organization leading task: University of North Carolina

Progress Metrics:

Deliverable(s):

Re: PREEMPT budget template and details

Richgels, Katherine L <krichgels@usgs.gov>

Mon 3/12/2018 4:58 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Hi Tonie,

The overhead calculation spreadsheet is attached.

Katie

On Mon, Mar 12, 2018 at 4:20 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Here's the budget template. Can you check the intranet and tell me the overhead rate? Thanks -Tonie
> ---------- Forwarded message ----------
> From: **Luke Hamel** <hamel@ecohealthalliance.org>
> Date: Wed, Mar 7, 2018 at 4:03 PM
> Subject: PREEMPT budget template and details
> To: "Rocke, Tonie" <trocke@usgs.gov>
> Cc: Rachel Abbott <rabbott@usgs.gov>, Aleksei Chmura <chmura@ecohealthalliance.org>, Evelyn Luciano <luciano@ecohealthalliance.org>, Jonathon Musser <musser@ecohealthalliance.org>, Alison Andre <andre@ecohealthalliance.org>, "Dr. Peter Daszak" <daszak@ecohealthalliance.org>
>
> Hi Tonie,
>
> Please see attached, a **PREEMPT budget template** for you to complete. Although this template lacks specific detail that will be required later, it will allow both you and our staff at EHA to determine a general budget breakdown for your institution.
>
> At the moment, please focus your attention on sections A-K, and fill out these sections as completely as possible. Section L (Budget justification) can be addressed at a later time, and I will be sure to provide you with additional instructions within the upcoming week.
>
> To assist you in completing the budget template, I have included the proposed disbursement schedule for the EHA-NWHC subcontract.
>
> | Year | Amount |
> |---|---|
> | Y1 | $304,500.00 |
> | Y2 | $304,500.00 |
> | OY1 | $304,500.00 |
> | OY .5 | $152,250.00 |
> | **Total** | **$1,065,750.00** |
>
> Lastly, please keep in mind the following items as you begin to determine specific aspects of your budget. Although this level of detail isn't needed for you to complete the attached budget template, it will be required for the final budget submission.
>
> Please let me know if you have any questions**. EHA staff will gladly assist you in procuring the necessary items, as required below.**
>
> 1) Regarding 'Indirect Costs':

If available, provide current Forward Pricing Rate Agreement or Forward Pricing Rate Proposal. If not available, provide 2 years historical data to include pool and expense costs used to generate the rates. For academia, provide DHHS or ONR negotiated rate package, or, if calculated by other than a rate, provide University documentation identifying G&A and fringe costs by position.

2) Regarding 'Travel':
Provide the purpose of the trip, number of trips, number of days per trip, departure and arrival destinations, number of people, estimated rental car and airfare costs, and prevailing per diem rates as determined by gsa.gov, etc.; Quotes must be supported by screenshots from travel websites.

3) Regarding 'Equipment Purchases'
Itemization with individual and total costs, including quantities, unit prices, proposed vendors (if known), and the basis of estimate (e.g., quotes, prior purchases, catalog price lists, etc.); any item that exceeds $5,000 must be supported with back-up documentation such as a copy of catalog price lists or quotes prior to purchase (NOTE: For equipment purchases, include a letter stating why the proposer cannot provide the requested resources from its own funding.

4) Regarding 'Materials'
Itemization with costs, including quantities, unit prices, proposed vendors (if known), and the basis of estimate (e.g., quotes, prior purchases, catalog price lists, etc.); any item that exceeds $5,000 must be supported with back-up documentation such as a copy of catalog price lists or quotes prior to purchase.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

--
Tonie E. Rocke
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Madison, WI 53711
608-270-2451
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--
Katherine L. D. Richgels, Ph.D.

Branch Chief, Applied Wildlife Health Research
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krichgels@usgs.gov
www.nwhc.usgs.gov

GROSS FUNDING			$78,030.00	
	64.543%	**46.017%**	**20.000%**	**17.919%**
NET	$47,422.25	$53,438.98	$65,025.00	$66,172.54
COM	$8,497.59	$4,007.92	$4,876.88	$11,857.46
FAC	$13,749.61	$15,494.10	$3,039.27	$0.00
BUREAU	$8,360.33	$5,089.09	$5,089.10	$0.00
TOTAL BURDEN COSTS	**$30,607.54**	**$24,591.12**	**$13,005.25**	**$11,857.46**

	TOTAL	COM	FAC

GROSS
COM
FAC
BUREAU
TOTAL BURDEN COSTS

BUR

Re: PREEMPT budget (follow-up)

Luke Hamel <hamel@ecohealthalliance.org>

Tue 3/13/2018 10:26 AM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>

Hi Tonie,

There was a typo in my previous email. When I referred to 'Adding budget periods' to the budget template, I spoke of a 'Year 3' for the project and said there were five budget periods. This is incorrect. **There are only 4 periods for the project and they are as follows: 'Year 1', 'Year 2', 'Option Year 1', 'Option Year .5'**. I apologize for the mistake.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Fri, Mar 9, 2018 at 7:23 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Hi Tonie,
>
> As you are filling out your PREEMPT budget, there are a few additional comments I'd like to make:
>
> - Regarding Section L, 'Budget Justification': Please begin drafting a **budget justification**, if you have not done so already. *This document must provide explanation for each line item within your detailed budget.* If you have any questions regarding this section, I'd be happy to provide you with additional information.
> - Adding budget periods: If you refer to the budget template I sent to you earlier this week, you'll see that the top of page 2 reads, 'Research and Related Budget- Budget Period 1'.
>   - This 'Budget Period 1' refers only to the first year of the proposed project. **In order to list expenses for subsequent years** (i.e. Year 2, Year 3, Option Year 1 and Option Year .5), you will have to **click the 'Add Period' button at the bottom of page 4** (just after Section L).
>   - You will then see a page that is titled, 'Research & Related Budget- Budget Period 2', with accompanying sections (A-L) that are identical to those in

Budget Period 1. Fill out this second budget period (copy over information from Budget Period 1 or edit as needed). You'll notice that the page titled, 'Research & Related Budget - Cumulative budget' updates as you add information to 'Budget Period 2.'

  - Repeat this process, adding periods for Year 3, Option Year 1 and Option Year .5
  - Attached to this email is a screenshot of the budget template document, highlighting where the 'Add Period' button can be found within the document.
- Budget 'Point of Contact': **Please let me know who is responsible for managing the PREEMPT budget at your institution**. We feel it will be easiest to communicate with this individual directly, to ensure nothing is lost when discussing budget-related items. If this individual is someone from another department at your institution, we will be sure to copy you on all emails.

Lastly, please return your budget (with all five 'budget periods' included) by Wed. 3/14, Eastern Time. We anticipate that you may have questions regarding certain aspects of the budget, so please do not hesitate to ask. We will try to resolve any issue as quickly as possible.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

[redacted] (direct)
(b) (6) (mobile)
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EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

Re: PREEMPT budget (follow-up)

Luke Hamel <hamel@ecohealthalliance.org>

Tue 3/13/2018 1:01 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Hi Tonie,

Yes, please include 'Indirect Costs' within Section H of the budget template. Also please note, that the total subcontract amount includes all costs (direct and indirect).

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Tue, Mar 13, 2018 at 12:45 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Thanks Luke: Were you expecting that our indirect costs were included in the proposed budget figures you gave me? Thanks -Tonie
>
> On Tue, Mar 13, 2018 at 10:26 AM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > There was a typo in my previous email. When I referred to 'Adding budget periods' to the budget template, I spoke of a 'Year 3' for the project and said there were five budget periods. This is incorrect. **There are only 4 periods for the project and they are as follows: 'Year 1', 'Year 2', 'Option Year 1', 'Option Year .5'**. I apologize for the mistake.
> >
> > Best,
> >
> > **Luke Hamel**
> > *Program Assistant*

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

[redacted] (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

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On Fri, Mar 9, 2018 at 7:23 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Hi Tonie,
>
> As you are filling out your PREEMPT budget, there are a few additional comments I'd like to make:
>
> - Regarding Section L, 'Budget Justification': Please begin drafting a **budget justification**, if you have not done so already. *This document must provide explanation for each line item within your detailed budget*. If you have any questions regarding this section, I'd be happy to provide you with additional information.
> - Adding budget periods: If you refer to the budget template I sent to you earlier this week, you'll see that the top of page 2 reads, 'Research and Related Budget- Budget Period 1'.
>   - This 'Budget Period 1' refers only to the first year of the proposed project. **In order to list expenses for subsequent years** (i.e. Year 2, Year 3, Option Year 1 and Option Year .5), you will have to **click the 'Add Period' button at the bottom of page 4** (just after Section L).
>   - You will then see a page that is titled, 'Research & Related Budget- Budget Period 2', with accompanying sections (A-L) that are identical to those in Budget Period 1. Fill out this second budget period (copy over information from Budget Period 1 or edit as needed). You'll notice that the page titled, 'Research & Related Budget - Cumulative budget' updates as you add information to 'Budget Period 2.'
>   - Repeat this process, adding periods for Year 3, Option Year 1 and Option Year .5
>   - Attached to this email is a screenshot of the budget template document, highlighting where the 'Add Period' button can be found within the document.
> - Budget 'Point of Contact': **Please let me know who is responsible for managing the PREEMPT budget at your institution**. We feel it will be easiest to communicate with this individual directly, to ensure nothing is lost when discussing budget-related items. If this individual is someone from another department at your institution, we will be sure to copy you on all emails.
>
> Lastly, please return your budget (with all five 'budget periods' included) by Wed. 3/14, Eastern Time. We anticipate that you may have questions regarding certain aspects of the budget, so please do not hesitate to ask. We will try to resolve any issue as quickly as possible.
>
> Best,

Feedback on PREEMPT graphic

Luke Hamel <hamel@ecohealthalliance.org>

Wed 3/14/2018 8:14 AM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Dr. Peter Daszak <daszak@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>

Hi Tonie,

As part of the PREEMPT proposal, we are required to include a graphic that illustrates our team's proposed technical approach. Below, I've attached a working draft of this graphic.

In our call today, we're hoping to briefly hear your thoughts about this image (especially in regards to the 'Field Testing and Deployment' panel). We will describe the graphic as it currently stands, then allow for your feedback.

Also, just a reminder that our call today is at 10 AM (ET):

Phone: 1-719-785-9461
Password: 9784#

Best,

Luke Hamel
Program Assistant

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460 West 34th Street – 17th floor
New York, NY 10001

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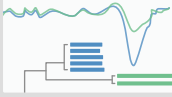
Host-Pathogen Prediction

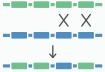
Host-Pathogen Niche Modeling

Assays in humanized mice



Machine learning genotype-phenotype mapping

Modeling recombination and evolution



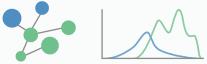
Validate models using human serology

Intervention Development

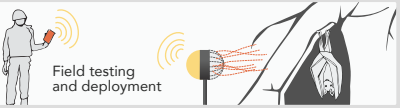
Immune boosting

Immune priming

Captive experiments



Simulating host-viral dynamics



Field testing and deployment

From: Rocke, Tonie <trocke@usgs.gov>
Sent: Tuesday, March 13, 2018 12:29 PM
To: Luke Hamel; Daszak Peter
Subject: Fwd: PARC FEA
Attachments: PARC_whitepaper_Biotech_v3_final.pdf

Hi Luke/Peter: In anticipation of our call tomorrow, take a look at the attached white paper and video on the link below. I think this looks like a great option for a spray device for bats, and it sounds like the material I have been working with already would work perfectly with this system. I haven't yet been able to pin them down on a price for a subcontract, but I'd like to talk to you tomorrow about this and some other budget details I am struggling with. Thanks -Tonie

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Subject: Re: PARC FEA
To: trocke@usgs.gov

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Please let me know how this might turn out.

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Jerome

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Innovative Solutions for Biotechnology @ PARC

Leveraging on our long history in fluid and particle manipulation and roll-to-roll processes for printing applications, the PARC hardware laboratories are currently developing innovative device solutions with potential applications in biomedicine and biotechnology at-large.

Filament Extension Atomizer (FEA)

PARC is developing a novel spray technology called the **Filament Extension Atomizer (FEA)** that can generate aerosol from fluids that are notoriously difficult to aerosolize due to the inherent *viscosity limit* with most conventional spray methods. FEA can spray fluids of a wide range of viscosities: from 1 mPa-s (the viscosity of water) up to 1000 Pa-s (the viscosity of peanut butter) – this range includes fluids or dispersions with significant (bio)macromolecular content. This macromolecular content (long chain polymers) often impart an additional resistance to aerosol/spray generation due to *strain hardening* or the increase of fluid viscosity as a function of extension.

To generate aerosol from such *strain hardening* fluids, FEA harnesses a well-known *elasto-capillary instability* that generates beads-on-a-string formation (Fig. 1A) when a fluid is held in extension. As the filaments are sufficiently thinned out, droplet break-up occurs and generates free droplets (aerosol). The FEA technology implements similar mechanics in a roll-to-roll process (Fig. 1B) to massively parallelize the filament formation and break-up and continuously generate droplets. To date, we have applied this on multiple viscoelastic fluids which include polymer solutions, polymer melts, particle dispersions and other complex systems with biomolecules (Figs. 1C-E).

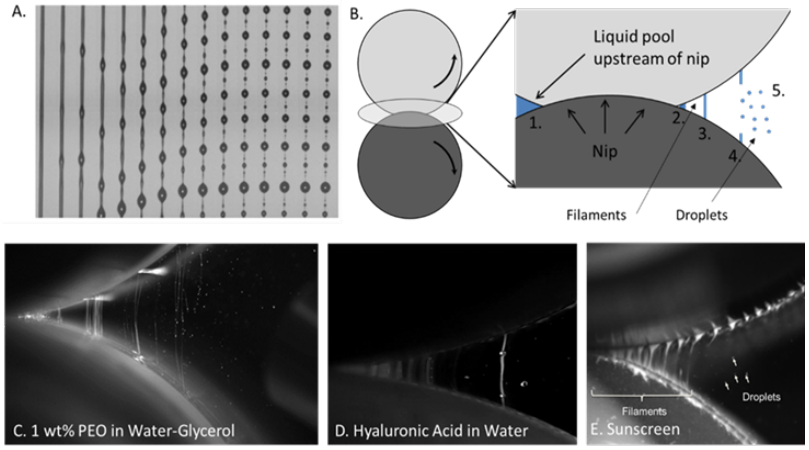
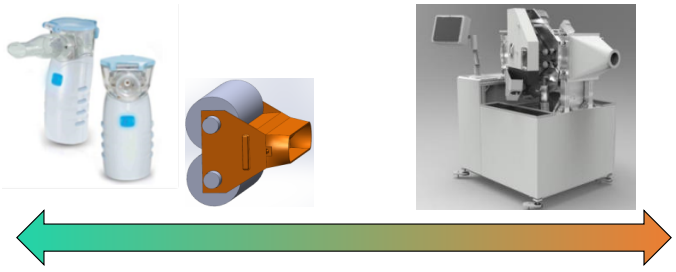


Fig. 1 – A. Beads-on-a-string structures in a viscoelastic fluid in extension (McKinley and Olivera, 2005), B. Multiple beads-on-a-string formations in counter-rotating rollers (FEA), FEA spraying of PEO solution (C.), hyaluronic acid (D.) and sunscreen (E.)



Consumer Scale
- Portable devices
- Small rollers (down to 10mm)
- Low throughput
- Precision fluid delivery (e.g. bioactive doses)
- Smart, connected devices

Industrial Scale
- Large rollers (up to 100mm)
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Fig. 2 – FEA Technology at Different Scales and Applications

The FEA technology is inherently scalable – it can work with small rollers for consumer scale applications, tailored for precision fluid dispensing, or with large rollers for industrial scale, high throughput aerosol generation for coatings or for powder production via spray drying. Since the FEA technology applies to wide range of fluids of virtually any viscosity or composition, it allows nearly formulation-independent fluid delivery. This can have a huge impact in biomedicine and biotechnology at-large: fluids can be *formulated for bio-efficacy* and not for delivery with no limits on bioactive loading, even with high viscosity biomacromolecules that are notoriously hard to deliver in a controlled, reliable manner. FEA can also allow the creation of specialized particles for drug delivery with a wide range of material set and control over the particle morphology.

PARC, 3333 Coyote Hill Road, Palo Alto, California 94304 USA +1 650 812 4000 | engage@parc.com | www.parc.com

Technical Contact:
Jerome Unidad (Jerome.Unidad@parc.com), Member of Research Staff

A global center for commercial innovation, PARC, a Xerox company, works closely with enterprises, entrepreneurs, government program partners and other clients to discover, develop, and deliver new business opportunities. PARC was incorporated in 2002 as a wholly owned subsidiary of Xerox Corporation (NYSE: XRX).

From: Jerome.Unidad@parc.com
Sent: Tuesday, March 13, 2018 11:54 AM
To: trocke@usgs.gov
Subject: Re: PARC FEA
Attachments: PARC_whitepaper_Biotech_v3_final.pdf

Tonie,

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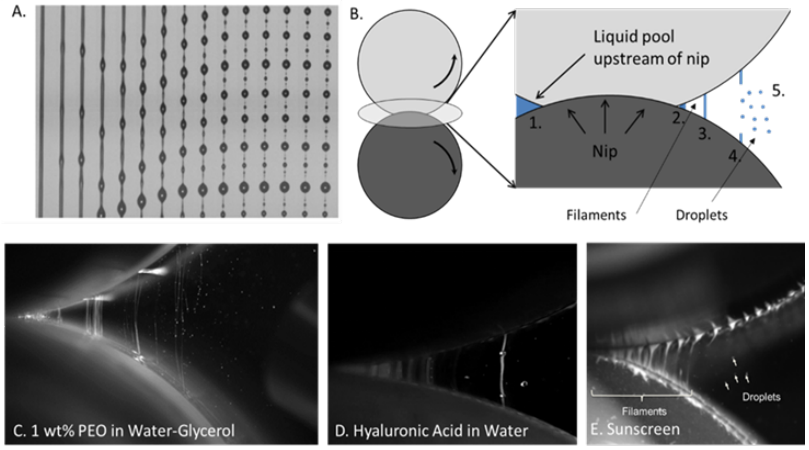

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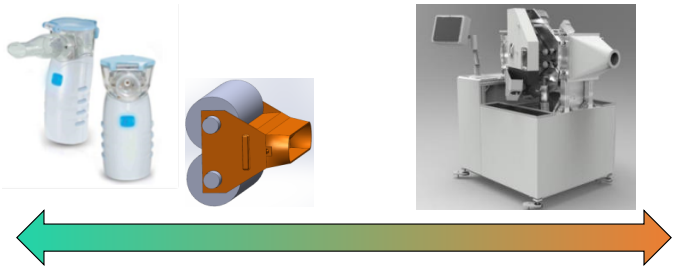

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Re: PARC FEA

William B. Karesh <(b) (6) @gmail.com>

Wed 3/14/2018 10:39 AM

To: Rocke, Tonie E <trocke@usgs.gov>

Thanks,

DARPA may also require EHA to get three quotes, though we can probably justify a sole-source agreement. But, in any case, it may be faster and easier for us to do it than the NWHC.

I'll flag that to Luke so he can make sure it ends up in the budget in the right place before submission.

BK

> On Mar 14, 2018, at 10:42 AM, Rocke, Tonie <trocke@usgs.gov> wrote:
>
> Here's the video we were talking about. I just had another thought after we hung up, that as a government agency, I would probably have to put the subcontract out for bid (unless I can write a really strong sole-source justification). But I suppose we can worry about all that and shift budget around if we get funding. Might be easier for EcoHealth Alliance to write the subcontract. I'll see what price I can get them down to. Best - Tonie
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Subject: Re:

Hi Jerome: 1-2 CT is fine for me. I may not be in my office, however, so can I call you? Is this number good? 650-812-4209. Thanks -Tonie

On Mon, Mar 12, 2018 at 8:34 PM, <Jerome.Unidad@parc.com> wrote:

> Sorry, I mixed up the schedule. I actually do have a meeting in the 1-2PM PST slot, how about 11AM-12PM PST (1-2PM CT)?
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> Thanks,
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> Jerome
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> **From:** "Rocke, Tonie" <trocke@usgs.gov>
> **Date:** Monday, March 12, 2018 at 5:25 PM
> **To:** "Unidad, Jerome <Jerome.Unidad@parc.com>"

<Jerome.Unidad@parc.com>
Subject: <no subject>

Hi Jerome: I have been working on developing vaccines for use in managing disease in wild bats - e.g. rabies in vampire bats and white-nose syndrome in insectiverous bats. I am also collaborating on a PREEMPT proposal and one of my collaborators passed on your contact information and description regarding PARC's spray technology for possible application in this field. Would you have time to chat tomorrow? I'll be available anytime after noon CT. Thanks much! - Tonie

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<PARC_whitepaper_Biotech_v3_final.pdf>

Re: PARC FEA

William B. Karesh <(b) (6) @gmail.com>

Wed 3/14/2018 12:28 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Just so you know for future thinking or planning in case Peter didn't already explain it.

Our NICRA rate is 31.5% this year, but for sub-awards like to you, it only applies to the first $25,000 and then 0% for the rest of the money in that contract (normally annual).

I'm not sure how it would apply for a large purchase order if we went that way with PARC. They may be okay with a sub-award, in which case the 25K limit would save us all money.

BK

On Mar 14, 2018, at 11:52 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

I suspect your OH rate is not as high as ours either. -T

On Wed, Mar 14, 2018 at 10:39 AM, William B. Karesh <(b) (6) @gmail.com> wrote:

Thanks,

DARPA may also require EHA to get three quotes, though we can probably justify a sole-source agreement. But, in any case, it may be faster and easier for us to do it than the NWHC.

I'll flag that to Luke so he can make sure it ends up in the budget in the right place before submission.

BK

On Mar 14, 2018, at 10:42 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

Here's the video we were talking about. I just had another thought after we hung up, that as a government agency, I would probably have to put the subcontract out for bid (unless I can write a really strong sole-source justification). But I suppose we can worry about all that and shift budget around if we get funding. Might be easier for EcoHealth Alliance to write the subcontract. I'll see what price I can get them down to. Best -Tonie

---------- Forwarded message ----------
From: **Rocke, Tonie** <trocke@usgs.gov>
Date: Tue, Mar 13, 2018 at 2:29 PM
Subject: Fwd: PARC FEA
To: Luke Hamel <hamel@ecohealthalliance.org>, Daszak Peter <daszak@ecohealthalliance.org>

Hi Luke/Peter: In anticipation of our call tomorrow, take a look at the attached white paper and video on the link below. I think this looks like a great option for a spray device for bats, and it sounds like the material I have been working with already would work perfectly with this system. I haven't yet been able to pin them down on a price for a subcontract, but I'd like to talk to you tomorrow about this and some other budget details I am struggling with. Thanks -Tonie

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From: <Jerome.Unidad@parc.com>
Date: Tue, Mar 13, 2018 at 1:53 PM
Subject: Re: PARC FEA
To: trocke@usgs.gov

Tonie,

Thanks for reaching out. Here's a 1-pager on our spray technology. If you are curious about how the spray might actually look like, you can check out a video here -- https://www.parc.com/services/focus-area/amds/

We would really be interested in working with your proposal team on this. If possible, it will be more value-generating for us to be a subcontractor and to contribute more to tailoring our spray technology for the intended use case. I'd also like to mention that another aspect we could bring to the table is in transitioning out the technology into a reality, particularly towards commercialization, because we have a good history on this – particularly on the device side but also, and increasingly, in the biomedical space through other commercial partners.

Please let me know how this might turn out.

Thanks,

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Date: Tuesday, March 13, 2018 at 5:11 AM
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RE: PARC FEA

Peter Daszak <daszak@ecohealthalliance.org>

Thu 3/15/2018 2:13 PM

To: Rocke, Tonie E <trocke@usgs.gov>; Billy Karesh <(b) (6) @gmail.com>; Luke Hamel <hamel@ecohealthalliance.org>

Tonie – Thanks for all of this. Re. PARC – Billy and I would like to have a quick call with Jerome. Would you like to join?

Either way, I'll send him an email now and try to set up a time this afternoon or tomorrow (Friday).

Bottom line – this is expensive, and if we're not exclusive with them, it's prob better to go with the cheaper option.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4474
www.ecohealthalliance.org
@PeterDaszak
@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 10:47 AM
To: Billy Karesh; Peter Daszak; Luke Hamel
Subject: Re: PARC FEA

A couple of other questions/comments. Are you proposing for co-PIs to meet periodically, perhaps at EHA? or elsewhere? Should we include that in our travel budgets? I think I heard yesterday someone from your shop was going to provide a budget for trips to China as well. Finally, just a heads up, I currently have a DOD SERDP grant and was caught by surprise how often they require the PI to travel to DC for progress reports and symposium (3 x last year, 2 x this year and the registration fee for each symposium is $1000), so be sure to include funds in your budget for that. Best -Tonie

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Phase II

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The cost of the entire involvement as drafted is $(b) (4) over the full period of 3.5 years (Phase I and II). Some of the tasks proposed above (example task 3) are included to ensure that we refine our technology continually to meet the requirements of the application at hand. We have a good working relationship with DARPA on various projects that we would want to maintain and, at the same time, we would like to take this chance to work with institutions such as yours on something with broad impact.

Please let me know what your thoughts are and whether this might work. I can make myself available for

a phone call tomorrow, as needed. Also, feel free to loop in your project PI/prime in the discussion in case it might be helpful.

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Date: Wednesday, March 14, 2018 at 10:45 AM
To: "Unidad, Jerome <Jerome.Unidad@parc.com>" <Jerome.Unidad@parc.com>
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> Jerome Unidad, PhD
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> Hardware Systems Laboratory
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Travel for DARPA

Peter Daszak <daszak@ecohealthalliance.org>

Thu 3/15/2018 2:14 PM

To: Rocke, Tonie E <trocke@usgs.gov>; Billy Karesh <(b) (6) @gmail.com>; Luke Hamel <hamel@ecohealthalliance.org>
Cc: Anna Willoughby <willoughby@ecohealthalliance.org>

Re. the travel – we're working out how many trips etc., but this needs to come out of your budget. We'll send info along to you v. soon.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
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Tel. +1 212-380-4474
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> > **Subject:** <no subject>
> >
> > Hi Jerome: I have been working on developing vaccines for use in managing disease in wild bats - e.g. rabies in vampire bats and white-nose syndrome in insectiverous bats. I am also collaborating on a PREEMPT proposal and one of my collaborators passed on your contact information and description regarding PARC's spray technology for possible application in this field. Would you have time to chat tomorrow? I'll be available anytime after noon CT. Thanks much! -Tonie
> >
> > --
> > Tonie E. Rocke

> > > USGS National Wildlife Health Center
> > > 6006 Schroeder Rd.
> > > Madison, WI 53711
> > > 608-270-2451
> > > trocke@usgs.gov

> > --
> > Tonie E. Rocke
> > USGS National Wildlife Health Center
> > 6006 Schroeder Rd.
> > Madison, WI 53711
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trocke@usgs.gov

RE: PARC FEA

Peter Daszak <daszak@ecohealthalliance.org>

Thu 3/15/2018 2:14 PM

To: Rocke, Tonie E <trocke@usgs.gov>; Billy Karesh <(b) (6) @gmail.com>; Luke Hamel <hamel@ecohealthalliance.org>

Tonie – re. the PARC budget, we'll put this in our central budget, so we reduce the overhead costs as you say below. If you can reduce your NWHC budget by $50K per year, to cover us for some of the costs.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4474
www.ecohealthalliance.org
@PeterDaszak
@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 10:47 AM
To: Billy Karesh; Peter Daszak; Luke Hamel
Subject: Re: PARC FEA

A couple of other questions/comments. Are you proposing for co-PIs to meet periodically, perhaps at EHA? or elsewhere? Should we include that in our travel budgets? I think I heard yesterday someone from your shop was going to provide a budget for trips to China as well. Finally, just a heads up, I currently have a DOD SERDP grant and was caught by surprise how often they require the PI to travel to DC for progress reports and symposium (3 x last year, 2 x this year and the registration fee for each symposium is $1000), so be sure to include funds in your budget for that. Best -Tonie

On Wed, Mar 14, 2018 at 8:21 PM, Rocke, Tonie <trocke@usgs.gov> wrote:
Hi all: Here's PARC's proposed "lean" budget ($(b) (4)) and it may be too expensive for us at this point (although to be honest I don't think it is overpriced). I tried to sell it as a way to illustrate the value of their technology, but it sounds like they already have a relationship with DARPA. Perhaps I just didn't sell it well enough. If either of you would like to try negotiating with them, feel free (bargaining is not

one of my strong suits!). One option might be to just get through #3 on their list for a total of $(b) (4) and then use the prototype developed for lab testing in the field (I'm assuming this will be a hand held device), and leave the field deployable unit for another grant; or maybe at that point DARPA would fund them directly. I can discuss this idea with him tomorrow. As Billy and I discussed today in any case, the subcontract should not come from USGS as it would be charged exorbitant overhead costs. If we decide to go this route, we can reduce the NWHC budget somewhat. Let me know what you think. Thanks! - Tonie

---------- Forwarded message ----------
From: <Jerome.Unidad@parc.com>
Date: Wed, Mar 14, 2018 at 5:46 PM
Subject: Re: PARC FEA
To: trocke@usgs.gov

Hi Tonie,

I agree based on our discussion that I think there's a lot of interesting space for our technology for wildlife health management that we can work on together. We are certainly interested in exploring these other funding opportunities with you, particularly the WNS one which seems to be in the intermediate term.

Regarding PRE-EMPT, we understand that coming in this late that you guys probably have already fleshed out the project direction and tasks with equivalent budget and there might not be a lot of flexibility. In our proposed involvement, we would not want to change anything that you already just fleshed but rather supplement it with our spray technology. Based on preliminary estimation on how this involvement might be like, I came up with the following tasks and a lean estimate of the associated cost:

Phase I

1. Development of a prototype FEA system for lab testing (Year 1) - $(b) (4)
2. Optimization of FEA spray conditions for PRE-EMPT fluids (Year 1) - $(b) (4)
3. Refinements of FEA delivery system (setup, fluid formulation, general aerosol delivery scheme) based on preliminary lab testing (Year 2) - $(b) (4)
4. Preliminary design of a field-deployable FEA system (Year 2) - $(b) (4)

Phase II

5. Fabrication and testing of field-deployable FEA systems (Year 3) - $(b) (4)
6. Project management and communication - $(b) (4)

The cost of the entire involvement as drafted is $(b) (4) over the full period of 3.5 years (Phase I and II). Some of the tasks proposed above (example task 3) are included to ensure that we refine our technology continually to meet the requirements of the application at hand. We have a good working relationship with DARPA on various projects that we would want to maintain and, at the same time, we would like to take this chance to work with institutions such as yours on something with broad impact.

Please let me know what your thoughts are and whether this might work. I can make myself available for a phone call tomorrow, as needed. Also, feel free to loop in your project PI/prime in the discussion in case it might be helpful.

Thanks,

RE: Rescheduling upcoming calls with Peter (PREEMPT)

Baric, Ralph S <rbaric@email.unc.edu>

Thu 3/15/2018 3:31 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Hi Tonie, I think I misunderstood RCN is the Raccoon poxvirus? Ralph

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Tuesday, March 13, 2018 1:19 PM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: Re: Rescheduling upcoming calls with Peter (PREEMPT)

Hi Ralph: Thanks for sending me your narrative. Just to clarify, are you proposing challenge trials in vaccinated bats at UNC? I think we should just subcontract with UW to engineer the RCN constructs. They are really skilled at it. We typically then produce the master seeds in my lab. I'm assuming those seeds would then go back to yours for challenge trials. Does that make sense? I'm trying to figure out how to prepare the budget. Same question I guess about the nanoparticles. Will you be constructing these in your lab and then testing them in bats? Thanks! -Tonie

On Sun, Mar 11, 2018 at 7:52 PM, Baric, Ralph S <rbaric@email.unc.edu> wrote:

> Its rough, but here's a draft. One section not included yet. Ralph
>
> **From:** Rocke, Tonie [mailto:trocke@usgs.gov]
> **Sent:** Friday, March 9, 2018 4:21 PM
> **To:** Luke Hamel <hamel@ecohealthalliance.org>
> **Cc:** Rachel Abbott <rabbott@usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Dr. Peter Daszak <daszak@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>
> **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)
>
> Hello Luke and Peter: Attached is my first draft of Task 7. Ralph Baric (copied here) and I had a good chat today about viral vectors and nanoparticles. We realized much of the delivery methods would depend on his work first, so there are some gaps here and the narrative will probably change after I see what Ralph has written. At any rate, this is something to at least start with. Have a good weekend! -Tonie
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> On Thu, Mar 8, 2018 at 12:58 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > Once again**, please use this link, to select which date(s)/time(s) you are available to speak with Peter (select as many as you are available for)**. The goal is to have one call each week, on either Tuesday or Wednesday. Below, I've listed the dates/times that appear in the Doodle Poll link above. Please note that all times listed are in Eastern Time.
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> > Thu. 3/21 (9 AM - 5 PM ET)

> Please let me know if you have any questions.
>
> Best,
>
> **Luke Hamel**
> *Program Assistant*
>
> EcoHealth Alliance
> 460 West 34th Street – 17th floor
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RE: Rescheduling upcoming calls with Peter (PREEMPT)

Baric, Ralph S <rbaric@email.unc.edu>

Thu 3/15/2018 3:39 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Ainslie, Kristy <ainsliek@email.unc.edu>

Hi Tonie, might be beneficial for you and Kristy to talk-who has nano/micro particle based delivery systems. I've included her email. Ralph

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Tuesday, March 13, 2018 1:19 PM
To: Baric, Ralph S <rbaric@email.unc.edu>
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> **Sent:** Friday, March 9, 2018 4:21 PM
> **To:** Luke Hamel <hamel@ecohealthalliance.org>
> **Cc:** Rachel Abbott <rabbott@usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Dr. Peter Daszak <daszak@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>
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Thu. 3/21 (9 AM - 5 PM ET)

Please let me know if you have any questions.

Best,

Luke Hamel
Program Assistant

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RE: Rescheduling upcoming calls with Peter (PREEMPT)

Baric, Ralph S <rbaric@email.unc.edu>

Thu 3/15/2018 3:50 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Okay, Peter said you should set up the subcontract through your section. Sorry for being dense. ralph

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 4:33 PM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: Re: Rescheduling upcoming calls with Peter (PREEMPT)

Yes it is. -T

On Thu, Mar 15, 2018 at 3:31 PM, Baric, Ralph S <rbaric@email.unc.edu> wrote:

> Hi Tonie, I think I misunderstood RCN is the Raccoon poxvirus? Ralph
>
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Please let me know if you have any questions.

Best,

Luke Hamel
Program Assistant

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RE: Rescheduling upcoming calls with Peter (PREEMPT)

Baric, Ralph S <rbaric@email.unc.edu>

Thu 3/15/2018 6:45 PM

To: Rocke, Tonie E <trocke@usgs.gov>

(b) (6) . morning is okay after 9am est.

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 7:28 PM
To: Ainslie, Kristy <ainsliek@email.unc.edu>
Cc: Baric, Ralph S <rbaric@email.unc.edu>
Subject: Re: Rescheduling upcoming calls with Peter (PREEMPT)

Tomorrow would probably work best for me as I need to get my part of this finished up soon. I will be heading out of the country on 3/22. If you give me a number I can call you. Thanks -Tonie

On Thu, Mar 15, 2018 at 5:14 PM, Ainslie, Kristy <ainsliek@email.unc.edu> wrote:

> Great! We could talk Friday 3/16 11-1, 3/19 8-1, or 3/21 8-2 all EST. Let me know a time that works for you.
>
> Thanks
> K
>
> **From:** Rocke, Tonie <trocke@usgs.gov>
> **Sent:** Thursday, March 15, 2018 6:12 PM
> **To:** Ainslie, Kristy <ainsliek@email.unc.edu>
> **Cc:** Baric, Ralph S <rbaric@email.unc.edu>
> **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)
>
> I am open to suggestions and actually would prefer you guys take that on. The project I mentioned is one I already have funded and I should have data by the time the funding for this would ever come in if it does. So we can always decide later. -T
>
> On Thu, Mar 15, 2018 at 5:05 PM, Ainslie, Kristy <ainsliek@email.unc.edu> wrote:
>
>> Tonie-
>>
>> Probably something similar. We can use PLGA, we often use our polymer acetalated dextran (works better outside the cold chain, acid-sensitive so better for immune cell uptake, better degradation properties, no acidic byproduct to degrade antigen etc.). Probably similar particles. Do you want to chat about this over the phone or are you going with the UW person?
>>
>> Thanks
>> K
>>
>> **From:** Rocke, Tonie <trocke@usgs.gov>
>> **Sent:** Thursday, March 15, 2018 5:06 PM
>> **To:** Baric, Ralph S <rbaric@email.unc.edu>
>> **Cc:** Ainslie, Kristy <ainsliek@email.unc.edu>
>> **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)

Hi Kristy: We have not tested anything in bats yet but will be soon. We are working with an engineer at UW, using PLGA to encapsulate rabies glycoprotein. What are you thinking of. -Tonie

On Thu, Mar 15, 2018 at 3:39 PM, Baric, Ralph S <rbaric@email.unc.edu> wrote:

> Hi Tonie, might be beneficial for you and Kristy to talk-who has nano/micro particle based delivery systems. I've included her email. Ralph
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Best,

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Program Assistant

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RE: Rescheduling upcoming calls with Peter (PREEMPT)

Baric, Ralph S <rbaric@email.unc.edu>

Fri 3/16/2018 8:44 AM

To: Rocke, Tonie E <trocke@usgs.gov>

Lets us talk whenever your ready. I'm at (b) (6) . ralph

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Friday, March 16, 2018 2:27 AM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: Re: Rescheduling upcoming calls with Peter (PREEMPT)

Hi Ralph: I can chat anytime before noon CT, and it is fine if Kristy joins us at the same time. It's really up to you. Best -Tonie

On Thu, Mar 15, 2018 at 8:30 PM, Baric, Ralph S <rbaric@email.unc.edu> wrote:

> Hi Tonie, I was definitely planning on testing whatever I could in mice, nanoparticles no problem but my understanding was that RCN doesn't work well in mice. I have no bat colony, no way for me to do the experiment-which I definitely think needs to be done or we have no credibility. My understanding another bat colony exists in China, but not sure who is doing what. Batized mice may be very expensive and limited in numbers, I have no details but thik they use immune deficient mice, must repopulate immune cells with primary bat immune cells derived from live bats…bet they can't get to many batized mice/bat, but I'm not sure. Its very limited when you humanize mice, 12=15 animals/donor. If he has genetically engineered batized mice, then more might be available.
>
> Was Kristy going to be part of our call tomorrow? If so, I can set up a conference number, but need to know times. If its just us, no need to set this up. let me know.
>
> **From:** Rocke, Tonie [mailto:trocke@usgs.gov]
> **Sent:** Thursday, March 15, 2018 8:16 PM
> **To:** Baric, Ralph S <rbaric@email.unc.edu>
> **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)
>
> Yeah, I'm figuring my budget out now, and I think I may be able to horsetrade with Jorge a little. He wants to contract with me to do a plague challenge, so I think he might be willing to make a single construct for us no charge (more than that I'm not sure) and it will be a wash and easier for everybody not to exchange $. What I am more worried about right now is which animal studies we are doing. Peter suggested the other day that we do all the bat studies. That's what I'd like to chat with you about. Once the RCN construct is made and the nanoparticles are produced, who is testing them for their efficacy in bats to determine the most likely products to use. Or is that all just being done in mice? (One possibility might be to do it in batized mice- that would be a hell of a lot cheaper than bats!) Most of my budget is going to testing the medium and methods of delivery to bats; assessing uptake in bats with biomarker studies, and contracting with a company to design a prototype machine for spraying the bats. Maybe it is clear in your mind and I have just been out of the loop, but in any case, we can discuss all that tomorrow morning. Have a good night. -Tonie
>
> On Thu, Mar 15, 2018 at 6:39 PM, Baric, Ralph S <rbaric@email.unc.edu> wrote:
>
> > I'd just flat out tell Peter that you need X more dollars in the budget. How much money do you imagine this will cost? Ralph

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 4:58 PM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: Re: Rescheduling upcoming calls with Peter (PREEMPT)

I'm not certain I am going to have enough funding for that in my budget, but I'll try to figure it out. My biggest problem is our facility has a ridiculous overhead rate that would even apply to subcontracts. -T

On Thu, Mar 15, 2018 at 3:50 PM, Baric, Ralph S <rbaric@email.unc.edu> wrote:

> Okay, Peter said you should set up the subcontract through your section. Sorry for being dense. ralph
>
> **From:** Rocke, Tonie [mailto:trocke@usgs.gov]
> **Sent:** Thursday, March 15, 2018 4:33 PM
> **To:** Baric, Ralph S <rbaric@email.unc.edu>
> **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)
>
> Yes it is. -T
>
> On Thu, Mar 15, 2018 at 3:31 PM, Baric, Ralph S <rbaric@email.unc.edu> wrote:
>
> > Hi Tonie, I think I misunderstood RCN is the Raccoon poxvirus? Ralph
> >
> > **From:** Rocke, Tonie [mailto:trocke@usgs.gov]
> > **Sent:** Tuesday, March 13, 2018 1:19 PM
> > **To:** Baric, Ralph S <rbaric@email.unc.edu>
> > **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)
> >
> > Hi Ralph: Thanks for sending me your narrative. Just to clarify, are you proposing challenge trials in vaccinated bats at UNC? I think we should just subcontract with UW to engineer the RCN constructs. They are really skilled at it. We typically then produce the master seeds in my lab. I'm assuming those seeds would then go back to yours for challenge trials. Does that make sense? I'm trying to figure out how to prepare the budget. Same question I guess about the nanoparticles. Will you be constructing these in your lab and then testing them in bats? Thanks! -Tonie
> >
> > On Sun, Mar 11, 2018 at 7:52 PM, Baric, Ralph S <rbaric@email.unc.edu> wrote:
> >
> > > Its rough, but here's a draft. One section not included yet. Ralph
> > >
> > > **From:** Rocke, Tonie [mailto:trocke@usgs.gov]
> > > **Sent:** Friday, March 9, 2018 4:21 PM
> > > **To:** Luke Hamel <hamel@ecohealthalliance.org>
> > > **Cc:** Rachel Abbott <rabbott@usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Dr. Peter Daszak <daszak@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>
> > > **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)
> > >
> > > Hello Luke and Peter: Attached is my first draft of Task 7. Ralph Baric (copied here) and I had a good chat today about viral vectors and nanoparticles. We realized much of the delivery methods would depend on his work first, so there are some gaps here and the narrative will probably change after I see what Ralph has written. At any rate, this is something to at least start with. Have a good weekend! -Tonie
> > >
> > > On Thu, Mar 8, 2018 at 12:58 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

Hi Tonie,

Once again**, please use this link, to select which date(s)/time(s) you are available to speak with Peter (select as many as you are available for)**. The goal is to have one call each week, on either Tuesday or Wednesday. Below, I've listed the dates/times that appear in the Doodle Poll link above. Please note that all times listed are in Eastern Time.

Week 1:
Thu. 3/13 (9 AM - 5 PM ET)
Thu. 3/14 (9 AM - 5 PM ET)

Week 2:
Thu. 3/20 (9 AM - 5 PM ET)
Thu. 3/21 (9 AM - 5 PM ET)

Please let me know if you have any questions.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

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> > > --
> > > Tonie E. Rocke
> > > USGS National Wildlife Health Center
> > > 6006 Schroeder Rd.
> > > Madison, WI 53711
> > > 608-270-2451
> > > trocke@usgs.gov

> > --
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trocke@usgs.gov

RE: Rescheduling upcoming calls with Peter (PREEMPT)

Ainslie, Kristy <ainsliek@email.unc.edu>

Fri 3/16/2018 10:03 AM

To: Rocke, Tonie E <trocke@usgs.gov>

T-

Here is the wiki article: https://en.wikipedia.org/wiki/Acetalated_dextran

Thanks
K

From: Rocke, Tonie <trocke@usgs.gov>
Sent: Friday, March 16, 2018 10:29 AM
To: Ainslie, Kristy <ainsliek@email.unc.edu>
Subject: Re: Rescheduling upcoming calls with Peter (PREEMPT)

Ha, I can relate. OK, I'll call in about 5 minutes or so (I need another cup of coffee!) -Tonie

On Fri, Mar 16, 2018 at 9:26 AM, Ainslie, Kristy <ainsliek@email.unc.edu> wrote:

> T-
>
> Yeah you can call any time, but my husband took my cell (long story). Can you call me on my work phone 919-962-4556.
>
> Thanks
> K
>
> **From:** Rocke, Tonie <trocke@usgs.gov>
> **Sent:** Friday, March 16, 2018 10:25 AM
> **To:** Ainslie, Kristy <ainsliek@email.unc.edu>
> **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)
>
> Hi Kristy: I can talk to you anytime it is convenient before 11 CT. Just let me know what works best for you, and I'll give you a ring. Ralph and I just had a chat. Thanks -Tonie
>
> On Thu, Mar 15, 2018 at 7:47 PM, Ainslie, Kristy <ainsliek@email.unc.edu> wrote:
>
> > Sure, my cell is (b) (6) . If you could give me an approximate time that would be helpful so I'm not off somewhere away from my phone.
> >
> > sent via phone.
> > Email: ainsliek@email.unc.edu
> > Website: ainslielab.web.unc.edu
> >
> > ---
> >
> > **From:** Rocke, Tonie <trocke@usgs.gov>
> > **Sent:** Thursday, March 15, 2018 7:27:40 PM
> > **To:** Ainslie, Kristy
> > **Cc:** Baric, Ralph S
> > **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)

Tomorrow would probably work best for me as I need to get my part of this finished up soon. I will be heading out of the country on 3/22. If you give me a number I can call you. Thanks -Tonie

On Thu, Mar 15, 2018 at 5:14 PM, Ainslie, Kristy <ainsliek@email.unc.edu> wrote:

> Great! We could talk Friday 3/16 11-1, 3/19 8-1, or 3/21 8-2 all EST. Let me know a time that works for you.
>
> Thanks
> K
>
> **From:** Rocke, Tonie <trocke@usgs.gov>
> **Sent:** Thursday, March 15, 2018 6:12 PM
> **To:** Ainslie, Kristy <ainsliek@email.unc.edu>
> **Cc:** Baric, Ralph S <rbaric@email.unc.edu>
> **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)
>
> I am open to suggestions and actually would prefer you guys take that on. The project I mentioned is one I already have funded and I should have data by the time the funding for this would ever come in if it does. So we can always decide later. -T
>
> On Thu, Mar 15, 2018 at 5:05 PM, Ainslie, Kristy <ainsliek@email.unc.edu> wrote:
>
> > Tonie-
> >
> > Probably something similar. We can use PLGA, we often use our polymer acetalated dextran (works better outside the cold chain, acid-sensitive so better for immune cell uptake, better degradation properties, no acidic byproduct to degrade antigen etc.). Probably similar particles. Do you want to chat about this over the phone or are you going with the UW person?
> >
> > Thanks
> > K
> >
> > **From:** Rocke, Tonie <trocke@usgs.gov>
> > **Sent:** Thursday, March 15, 2018 5:06 PM
> > **To:** Baric, Ralph S <rbaric@email.unc.edu>
> > **Cc:** Ainslie, Kristy <ainsliek@email.unc.edu>
> > **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)
> >
> > Hi Kristy: We have not tested anything in bats yet but will be soon. We are working with an engineer at UW, using PLGA to encapsulate rabies glycoprotein. What are you thinking of. -Tonie
> >
> > On Thu, Mar 15, 2018 at 3:39 PM, Baric, Ralph S <rbaric@email.unc.edu> wrote:
> >
> > > Hi Tonie, might be beneficial for you and Kristy to talk-who has nano/micro particle based delivery systems. I've included her email. Ralph
> > >
> > > **From:** Rocke, Tonie [mailto:trocke@usgs.gov]
> > > **Sent:** Tuesday, March 13, 2018 1:19 PM
> > > **To:** Baric, Ralph S <rbaric@email.unc.edu>
> > > **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)
> > >
> > > Hi Ralph: Thanks for sending me your narrative. Just to clarify, are you proposing challenge trials in vaccinated bats at UNC? I think we should just subcontract with UW to engineer the RCN

constructs. They are really skilled at it. We typically then produce the master seeds in my lab. I'm assuming those seeds would then go back to yours for challenge trials. Does that make sense? I'm trying to figure out how to prepare the budget. Same question I guess about the nanoparticles. Will you be constructing these in your lab and then testing them in bats? Thanks! -Tonie

On Sun, Mar 11, 2018 at 7:52 PM, Baric, Ralph S <rbaric@email.unc.edu> wrote:

> Its rough, but here's a draft. One section not included yet. Ralph
>
> **From:** Rocke, Tonie [mailto:trocke@usgs.gov]
> **Sent:** Friday, March 9, 2018 4:21 PM
> **To:** Luke Hamel <hamel@ecohealthalliance.org>
> **Cc:** Rachel Abbott <rabbott@usgs.gov>; Aleksei Chmura <chmura@ecohealthalliance.org>; Dr. Peter Daszak <daszak@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>
> **Subject:** Re: Rescheduling upcoming calls with Peter (PREEMPT)
>
> Hello Luke and Peter: Attached is my first draft of Task 7. Ralph Baric (copied here) and I had a good chat today about viral vectors and nanoparticles. We realized much of the delivery methods would depend on his work first, so there are some gaps here and the narrative will probably change after I see what Ralph has written. At any rate, this is something to at least start with. Have a good weekend! -Tonie
>
> On Thu, Mar 8, 2018 at 12:58 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > Once again**, please use this link, to select which date(s)/time(s) you are available to speak with Peter (select as many as you are available for)**. The goal is to have one call each week, on either Tuesday or Wednesday. Below, I've listed the dates/times that appear in the Doodle Poll link above. Please note that all times listed are in Eastern Time.
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> >
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> > Thu. 3/21 (9 AM - 5 PM ET)
> >
> > Please let me know if you have any questions.
> >
> > Best,
> >
> > **Luke Hamel**
> > *Program Assistant*
> >
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Re: DARPA PRE-EMPT

Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>

Fri 3/16/2018 12:01 PM

To: karesh@ecohealthalliance.org <karesh@ecohealthalliance.org>
Cc: Rocke, Tonie E <trocke@usgs.gov>; David.Johnson@parc.com <David.Johnson@parc.com>; daszak@ecohealthalliance.org <daszak@ecohealthalliance.org>; hamel@ecohealthalliance.org <hamel@ecohealthalliance.org>; willoughby@ecohealthalliance.org <willoughby@ecohealthalliance.org>; andre@ecohealthalliance.org <andre@ecohealthalliance.org>; amanda.andre@ecohealthalliance.org <amanda.andre@ecohealthalliance.org>

Clarification to everyone:

Our call in line is: 1-719-785-9461
Passcode: 9784#

we're using EcoHealth's call-in line.

Thanks,

Jerome

From: "William B. Karesh" <karesh@ecohealthalliance.org>
Date: Thursday, March 15, 2018 at 4:13 PM
To: "Unidad, Jerome <Jerome.Unidad@parc.com>" <Jerome.Unidad@parc.com>
Cc: "Rocke, Tonie" <trocke@usgs.gov>, "Johnson, David <David.Johnson@parc.com>" <David.Johnson@parc.com>, Peter Daszak <daszak@ecohealthalliance.org>, Luke Hamel <hamel@ecohealthalliance.org>, Anna Willoughby <willoughby@ecohealthalliance.org>, Alison Andre <andre@ecohealthalliance.org>, Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

Our call in line is: 1-719-785-9461
Passcode: 9784#

Re: DARPA PRE-EMPT

Anna Willoughby <willoughby@ecohealthalliance.org>

Fri 3/16/2018 2:12 PM

To: Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Peter Daszak <daszak@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>; Luke Hamel <hamel@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Fuchs <amanda.andre@ecohealthalliance.org>; Kateri.Paul@parc.com <Kateri.Paul@parc.com>

Thanks for these details, Jerome. Attached are my notes from the call. Action items include:

- Jerome to send more detailed scope of work with paragraphs and revised budget by early next week
- EHA will send PARC the NWHC section of the proposal on Monday
- EHA will send the format of letter of support for PARC
- EHA to follow up with Kateri with requested information

For your question on collaborating with other institutes, it is likely that all organizations involved may have insight into the aerosol-bat interaction. I believe this topic would be covered during the Annual Meeting between all partners, as well as during relevant cross-partner trips, in addition to monthly conference calls.

Please let us know if you have further questions.

Best,
Anna

On Fri, Mar 16, 2018 at 2:57 PM, <Jerome.Unidad@parc.com> wrote:

> An additional point for Peter, Tonie (and everyone),
>
> For the spray technology, refinement of the details with respect to aerosol-bat interaction (i.e. the preliminary field testing to see how bats react to the aerosol) and eventual field-deployment in China, will the technical lead for coordinating this segment of the project be USGS – National Wildlife Center? Or should we also expect to work/coordinate with other institutes who would give feedback and insights on how this works?
>
> Thanks. This is just for our information.

Best,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Unidad, Jerome <Jerome.Unidad@parc.com>
Sent: Friday, March 16, 2018 11:52 AM
To: 'William B. Karesh' <karesh@ecohealthalliance.org>; 'Peter Daszak' <daszak@ecohealthalliance.org>
Cc: 'Luke Hamel' <hamel@ecohealthalliance.org>; 'Anna Willoughby' <willoughby@ecohealthalliance.org>; 'Alison Andre' <andre@ecohealthalliance.org>; 'Amanda Andre' <amanda.andre@ecohealthalliance.org>; 'Rocke, Tonie' <trocke@usgs.gov>; Paul, Kateri <Kateri.Paul@parc.com> <Kateri.Paul@parc.com>
Subject: RE: DARPA PRE-EMPT

Peter and team,

I'm currently working on putting together a revised budget and equivalent statement of work (tasks breakdown) for PARC's involvement with the project. You can expect this about early next week – approximately Monday. Officially, for the submission, our capture manager, Kateri Paul, who takes care of the other things would need the following things from your equivalent to facilitate our parts of the submission.

1. Request for Proposal that we can respond to with what they need for their package to DARPA
2. Start date of the proposed effort
3. Contract or a Grant/Other Transaction

Once we have finalized the scope of work and the budget, Kateri will be in touch for these other aspects. Her contact information can be found below.

Kateri E. Paul

Capture Manager, Public Sector

Global Business Development

Palo Alto Research Center (PARC)

3333 Coyote Hill Road

Palo Alto, CA 94304

Kateri.Paul@parc.com

650-812-4821 (desk)

617-596-2023 (mobile)

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Unidad, Jerome <Jerome.Unidad@parc.com>
Sent: Thursday, March 15, 2018 3:33 PM
To: 'Rocke, Tonie' <trocke@usgs.gov>; William B. Karesh <karesh@ecohealthalliance.org>; Johnson, David <David.Johnson@parc.com> <David.Johnson@parc.com>
Cc: Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: RE: DARPA PRE-EMPT

Dear all,

10AM-11AM PST (12PM-1PM CT, 1PM-2PM ET) should work for us. I shall setup a WebEx meeting for this, given the number of participants.

Let me know if this timeslot will work.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 2:39 PM
To: William B. Karesh <karesh@ecohealthalliance.org>
Cc: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I assume that is ET? -T

On Thu, Mar 15, 2018 at 4:14 PM, William B. Karesh <karesh@ecohealthalliance.org> wrote:

> Tonie and Jerome,
>
> We would still like to speak. Anytime on Friday between 11:00 AM and 2:00 PM would be great.
>
> BK
>
> **William B. Karesh, D.V.M**
>
> *Executive Vice President for Health and Policy*
>
> EcoHealth Alliance
>
> 460 West 34th Street - 17th Floor
>
> New York, NY 10001 USA
>
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President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

On Mar 15, 2018, at 4:55 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

Hi all: Since we didn't hear back from EcoHealth Alliance, Jerome and I went ahead with a short call we had been planning anyway regarding some technical details. I told him our concerns about the proposed budget and we think we have a pretty good plan to reduce the scope of work to the funds we have available. PARC is very unique in developing this technology and their technology fits very well with other work I am doing, so we both feel pretty confident we can work something out. If you still wish to have a discussion among all of us, we can schedule that for tomorrow, as I believe Jerome had another meeting to run off to for the rest of the day. I'm available the rest of the day if you wish to chat about this in person. Best -Tonie

On Thu, Mar 15, 2018 at 3:42 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

> Actually – can we do a phone call – I'll be driving. 5.15pm would be perfect (NYC time), Today Thursday.

Is that possible?

Our call in line is: 1-719-785-9461

Passcode: 9784#

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4474

www.ecohealthalliance.org

@PeterDaszak

@EcoHealthNYC

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From: Jerome.Unidad@parc.com [mailto:Jerome.Unidad@parc.com]
Sent: Thursday, March 15, 2018 4:23 PM
To: trocke@usgs.gov
Cc: William B. Karesh; Peter Daszak; Luke Hamel
Subject: RE: DARPA PRE-EMPT

I can setup a WebEx quickly if we will have multiple parties.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 1:22 PM
To: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Daszak Peter <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I'm available as well. Billy, do you have a call in number? -Tonie

On Thu, Mar 15, 2018 at 3:20 PM, <Jerome.Unidad@parc.com> wrote:

> Dear all,
>
> Sorry for the late response – yes, I will be available for a phone call now. Up to 2PM.
>
> Jerome
>
> ---

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: William B. Karesh [mailto:karesh@ecohealthalliance.org]
Sent: Thursday, March 15, 2018 12:49 PM
To: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
Cc: Rocke, Tonie <trocke@usgs.gov>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
Subject: DARPA PRE-EMPT

Dear Dr. Unidad,

Thanks for your quick responses to Dr. Rocke. Would you be available for a short call with Dr. Daszak, Dr. Rocke and me this afternoon or Friday.

We're on tight timeline so we thought a phone call might be save quite a bit of time.

Thanks in advance,

Billy

William B. Karesh, D.V.M

Executive Vice President for Health and Policy

EcoHealth Alliance

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Tonie E. Rocke

USGS National Wildlife Health Center

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trocke@usgs.gov

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Tonie E. Rocke

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Anna Willoughby
Research Assistant

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Visit our blog: http://blog.ecohealthalliance.org/updates

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March 15, 2018, 1pm EST
EHA: Billy Karesh, Peter Daszak, Anna WIlloughby
NWHC: Tonie Rocke
PARC: Jerome Unidad and David Johnson

Project DEFUSE, PI Peter Daszak

Budget

- Current budget is 580k for all tasks in original scope (360k development; 220 field prototype: 3-4 copies)
- EHA: Budget is currently too expensive. Avenues for reduction:
    - Reduce scope/trim intermediate steps? (not preferable)
- Original estimate is lean for prototype for scale: 2-3 bats at a time, generating aerosol for significant amount of space
- PARC may be able to make less expensive (for beginning scope of work)
- DARPA may have further, add-on support after program has begun
- Include some travel: PARC will need a cross-partner visit with EHA (or vice versa), will attend annual meeting, Y2 China visit, visit to TR captive colony in Y1.

Collaboration

- Exclusive partnership between EHA and PARC for DARPA application

Scope of Work (EHA needs more details, paragraph per item)

- Goals for EHA are to have engagement from PARC for duration of project and for deployment trials
- PARC sent white paper, should insert relevant info into proposal
- PARC want time to optimize correctly, (eg spray quality, fluid consistency)
- For DARPA purposes: proof of concept that you can interfere and disrupt v. transmission. Large scale intervention not necessary.
- Y1: Creating initial product, modifying existing fixtures; deploy biomarker in captive species, each captive bat experiment is ~32k (TR)
- Y2: Refining prototype for field use, deploy biomarker in field species stateside (TR)
    - Bats will be sampled for biomarker spread
- **EHA add link to video in proposal**

Deployment Details

- For Chinese bat caves: we would go to minor entrances/side pocket. (smaller scale, could then be scaled up after the project)
- PARC: How big are caves? EHA: Volume 2 ft by 2 ft, similar to furniture size, Not going to cave with 10,000 bats. This is simply a field trial.
- EHA: Deploy for 2-3 days at one site for field trial in China. Have at least 2 prototypes
- EHA: Will not manufacture large-scale spray material as too expensive
- Deploy Biomarker Study: Captive Bats (NWHC) -> Field (US) -> Field (China)
- Deploy Mesocosm Study: Captive Bats (Duke-NUS) -> Field (China)

-

Re: DARPA PRE-EMPT

Anna Willoughby <willoughby@ecohealthalliance.org>

Fri 3/16/2018 4:09 PM

To: Kateri.Paul@parc.com <Kateri.Paul@parc.com>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Peter Daszak <daszak@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>; Luke Hamel <hamel@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Fuchs <amanda.andre@ecohealthalliance.org>; Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>

Hi Kateri,

Yes, if possible we would appreciate a rapid return of the cost proposal. Monday is ideal, but Tuesday would be fine. Please find templates for materials and travel (which also outlines prospective travel). Did you receive the main budget template? If not, I am attaching a fresh one as well. Please let me know if you have any questions.

Thanks,
Anna

On Fri, Mar 16, 2018 at 4:27 PM, <Kateri.Paul@parc.com> wrote:

> Hello Anna,
>
> Could you please let me know your target deadline for our cost proposal? I understand it will be tight with the full proposal due 3/27.
>
> Thanks!
>
> Kateri
>
> **From:** Anna Willoughby [mailto:willoughby@ecohealthalliance.org]
> **Sent:** Friday, March 16, 2018 12:12 PM
> **To:** Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
> **Cc:** William B. Karesh <karesh@ecohealthalliance.org>; Peter Daszak <daszak@ecohealthalliance.org>; trocke@usgs.gov; Luke Hamel <hamel@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Fuchs <amanda.andre@ecohealthalliance.org>; Paul, Kateri <Kateri.Paul@parc.com> <Kateri.Paul@parc.com>
> **Subject:** Re: DARPA PRE-EMPT
>
> Thanks for these details, Jerome. Attached are my notes from the call. Action items include:
>
> *- Jerome to send more detailed scope of work with paragraphs and revised budget by early next week*
>
> *- EHA will send PARC the NWHC section of the proposal on Monday*
> *- EHA will send the format of letter of support for PARC*
> *- EHA to follow up with Kateri with requested information*

For your question on collaborating with other institutes, it is likely that all organizations involved may have insight into the aerosol-bat interaction. I believe this topic would be covered during the Annual Meeting between all partners, as well as during relevant cross-partner trips, in addition to monthly conference calls.

Please let us know if you have further questions.

Best,
Anna

On Fri, Mar 16, 2018 at 2:57 PM, <Jerome.Unidad@parc.com> wrote:

> An additional point for Peter, Tonie (and everyone),
>
> For the spray technology, refinement of the details with respect to aerosol-bat interaction (i.e. the preliminary field testing to see how bats react to the aerosol) and eventual field-deployment in China, will the technical lead for coordinating this segment of the project be USGS – National Wildlife Center? Or should we also expect to work/coordinate with other institutes who would give feedback and insights on how this works?
>
> Thanks. This is just for our information.
>
> Best,
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company
>
> ---
>
> **From:** Unidad, Jerome <Jerome.Unidad@parc.com>
> **Sent:** Friday, March 16, 2018 11:52 AM
> **To:** 'William B. Karesh' <karesh@ecohealthalliance.org>; 'Peter Daszak' <daszak@ecohealthalliance.org>
> **Cc:** 'Luke Hamel' <hamel@ecohealthalliance.org>; 'Anna Willoughby' <willoughby@ecohealthalliance.org>; 'Alison Andre' <andre@ecohealthalliance.org>; 'Amanda Andre' <amanda.andre@ecohealthalliance.org>;

'Rocke, Tonie' <trocke@usgs.gov>; Paul, Kateri <Kateri.Paul@parc.com> <Kateri.Paul@parc.com>
Subject: RE: DARPA PRE-EMPT

Peter and team,

I'm currently working on putting together a revised budget and equivalent statement of work (tasks breakdown) for PARC's involvement with the project. You can expect this about early next week – approximately Monday. Officially, for the submission, our capture manager, Kateri Paul, who takes care of the other things would need the following things from your equivalent to facilitate our parts of the submission.

1. Request for Proposal that we can respond to with what they need for their package to DARPA
2. Start date of the proposed effort
3. Contract or a Grant/Other Transaction

Once we have finalized the scope of work and the budget, Kateri will be in touch for these other aspects. Her contact information can be found below.

Kateri E. Paul

Capture Manager, Public Sector

Global Business Development

Palo Alto Research Center (PARC)

3333 Coyote Hill Road

Palo Alto, CA 94304

Kateri.Paul@parc.com

650-812-4821 (desk)

617-596-2023 (mobile)

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Unidad, Jerome <Jerome.Unidad@parc.com>
Sent: Thursday, March 15, 2018 3:33 PM
To: 'Rocke, Tonie' <trocke@usgs.gov>; William B. Karesh <karesh@ecohealthalliance.org>; Johnson, David <David.Johnson@parc.com> <David.Johnson@parc.com>
Cc: Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: RE: DARPA PRE-EMPT

Dear all,

10AM-11AM PST (12PM-1PM CT, 1PM-2PM ET) should work for us. I shall setup a WebEx meeting for this, given the number of participants.

Let me know if this timeslot will work.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 2:39 PM
To: William B. Karesh <karesh@ecohealthalliance.org>
Cc: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I assume that is ET? -T

On Thu, Mar 15, 2018 at 4:14 PM, William B. Karesh <karesh@ecohealthalliance.org> wrote:

Tonie and Jerome,

We would still like to speak. Anytime on Friday between 11:00 AM and 2:00 PM would be great.

BK

William B. Karesh, D.V.M

Executive Vice President for Health and Policy

EcoHealth Alliance

460 West 34th Street - 17th Floor

New York, NY 10001 USA

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President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

On Mar 15, 2018, at 4:55 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

Hi all: Since we didn't hear back from EcoHealth Alliance, Jerome and I went ahead with a short call we had been planning anyway regarding some technical details. I told him our concerns about the proposed budget and we think we have a pretty good plan to reduce the scope of work to the funds we have available. PARC is very unique in developing this technology and their technology fits very well with other work I am doing, so we both feel pretty confident we can work something out. If you still wish to have a discussion among all of us, we can schedule that for tomorrow, as I believe Jerome had another meeting to run off to for the rest of the day. I'm available the rest of the day if you wish to chat about this in person. Best -Tonie

On Thu, Mar 15, 2018 at 3:42 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

> Actually – can we do a phone call – I'll be driving. 5.15pm would be perfect (NYC time), Today Thursday.
>
> Is that possible?
>
> Our call in line is: 1-719-785-9461
>
> Passcode: 9784#
>
> Cheers,
>
> Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4474

www.ecohealthalliance.org

@PeterDaszak

@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Jerome.Unidad@parc.com [mailto:Jerome.Unidad@parc.com]
Sent: Thursday, March 15, 2018 4:23 PM
To: trocke@usgs.gov
Cc: William B. Karesh; Peter Daszak; Luke Hamel
Subject: RE: DARPA PRE-EMPT

I can setup a WebEx quickly if we will have multiple parties.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 1:22 PM
To: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Daszak Peter <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I'm available as well. Billy, do you have a call in number? -Tonie

On Thu, Mar 15, 2018 at 3:20 PM, <Jerome.Unidad@parc.com> wrote:

> Dear all,
>
> Sorry for the late response – yes, I will be available for a phone call now. Up to 2PM.
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company
>
> ---
>
> **From:** William B. Karesh [mailto:karesh@ecohealthalliance.org]
> **Sent:** Thursday, March 15, 2018 12:49 PM
> **To:** Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
> **Cc:** Rocke, Tonie <trocke@usgs.gov>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
> **Subject:** DARPA PRE-EMPT
>
> Dear Dr. Unidad,

Thanks for your quick responses to Dr. Rocke. Would you be available for a short call with Dr. Daszak, Dr. Rocke and me this afternoon or Friday.

We're on tight timeline so we thought a phone call might be save quite a bit of time.

Thanks in advance,

Billy

William B. Karesh, D.V.M

Executive Vice President for Health and Policy

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ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

--

Tonie E. Rocke

USGS National Wildlife Health Center

6006 Schroeder Rd.

Madison, WI 53711

608-270-2451

trocke@usgs.gov

--

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--

Anna Willoughby
Research Assistant

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Visit our blog: http://blog.ecohealthalliance.org/updates

--

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Item	Manufacturer	Part Number
Computers	Apple	13-inch MacBook Air
Monitors	Apple	Thunderbolt Display

Note:
Consumables may be listed as a lump sum if no individual item is over $5,000. For those items that a

MATERIALS/EQUIPMENT

Unit Price	Quantity	Total Price	Contract Period
$1,981	2	$3,962	Base Period
$ 1,098.00	3	$3,294	Base Period
		$7,256	

ıre over $5,000, list separately from the rest of consumable pricing

Additional Information
Current price on apple.com, including upgraded CPU, memory, and storage (http://store.ap
Current price on apple.com (http://store.apple.com/us/product/MC914LL/B/apple-thunderb

.

ple.com/us/buy-mac/macbook-air) Part #Z0P0
olt-display-27-inch?fnode=53) Part # MC914LL/B

Description	Total Price	Contract Period
	$0	

OTHER DIRECT COSTS

Additional Information

TRAVEL

Trip #:	1	Location:	Madison, WI
Purpose:	Partner Visit to National Wildlife Health Center		

Days	# of People	Airfare	Per Diem	Lodging
3	2			

Itemized Expenses for "Other"

Description	Amount
Transportation to/from airport and in Madison	$120.00
Total:	$120.00

Trip #:	2	Location:	New York, NY
Purpose:	Annual Meeting		

Days	# of People	Airfare	Per Diem	Lodging
3	1			

Itemized Expenses for "Other"

Description	Amount
Transportation to/from airport and in New York	
Total:	$0.00

Trip #:	3	Location:	Wuhan, China
Purpose:	Site Visit at Field Site		

Days	# of People	Airfare	Per Diem	Lodging
6	2			

Itemized Expenses for "Other"

Description	Amount
Transportation to/from airport and in Wuhan	
Total:	$0.00

Trip #:	4	Location:	Wuhan, China
Purpose:	Annual Meeting		

Days	# of People	Airfare	Per Diem	Lodging
3	1			

Itemized Expenses for "Other"

Description	Amount
Transportation to/from airport and in Wuhan	
Total:	$0.00

Trip #:	5	Location:	New York, NY
Purpose:	Annual Meeting		

Days	# of People	Airfare	Per Diem	Lodging
3	1			

Itemized Expenses for "Other"

Description	Amount
Transportation to/from airport and in New York	
Total:	$0.00

Trip #:	6	**Location:**	New York, NY
Purpose:	Annual Meeting		

Days	# of People	Airfare	Per Diem	Lodging
3	1			

Itemized Expenses for "Other"

Description	Amount
Transportation to/from airport and in New York	
Total:	$0.00

Contract Period	
Base Period	
Other	**Total**

Contract Period	
Base Period	
Other	**Total**

Contract Period	
Base Period	
Other	**Total**
	$0.00

Contract Period	
Base Period	
Other	**Total**

Contract Period
Option I

Other	Total
	$0.00

Contract Period
Option II

Other	Total

WORKSPACE FORM

1-800-518-4726
SUPPORT@GRANTS.GOV

This Workspace form is one of the forms you need to complete prior to submitting your Application Package. This form can be completed in its entirety offline using Adobe Reader. You can save your form by clicking the "Save" button and see any errors by clicking the “Check For Errors” button. In-progress and completed forms can be uploaded at any time to Grants.gov using the Workspace feature.

When you open a form, required fields are highlighted in yellow with a red border. Optional fields and completed fields are displayed in white. If you enter invalid or incomplete information in a field, you will receive an error message. Additional instructions and FAQs about the Application Package can be found in the Grants.gov Applicants tab.

OPPORTUNITY & PACKAGE DETAILS:

Opportunity Number:	HR001118S0017
Opportunity Title:	PREventing EMerging Pathogenic Threats
Opportunity Package ID:	PKG00237724
CFDA Number:	12.910
CFDA Description:	Research and Technology Development
Competition ID:	
Competition Title:	
Opening Date:	01/19/2018
Closing Date:	03/27/2018
Agency:	DARPA - Biological Technologies Office
Contact Information:	BAA Coordinator PREEMPT@darpa.mil

APPLICANT & WORKSPACE DETAILS:

Workspace ID:	WS00094394
Application Filing Name:	Project DEFUSE
DUNS:	0770900660000
Organization:	ECOHEALTH ALLIANCE INC.
Form Name:	R & R Subaward Budget 10 YR Subform
Form Version:	1.4
Subform Name:	Guangjian Zhu (consultant)
Requirement:	Optional
Download Date/Time:	Mar 15, 2018 05:50:37 PM EDT
Form State:	Error(s)

FORM ACTIONS:

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0000000000000 **Enter name of Organization:** PARC (consultant)

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 1** **Start Date:** 12/01/2018 **End Date:** 11/30/2020

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)

Project Role: PD/PI

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						

Total Number Other Personnel

Total Other Personnel

Total Salary, Wages and Fringe Benefits (A+B)

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.		
9.		
10.		
	Total Other Direct Costs	

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total Direct Costs			

Total Indirect Costs

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	

L. Budget Justification

(Only attach one file.) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0000000000000 **Enter name of Organization:** PARC (consultant)

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 2** **Start Date:** 12/01/2020 **End Date:** 05/31/2022

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)

Project Role: PD/PI

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						

Total Number Other Personnel

Total Other Personnel

Total Salary, Wages and Fringe Benefits (A+B)

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.		
9.		
10.		
	Total Other Direct Costs	

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total Direct Costs			

Total Indirect Costs

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	

L. Budget Justification

(Only attach one file.) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		
1. Materials and Supplies		
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		
Section H, Indirect Costs		
Section I, Total Direct and Indirect Costs (G + H)		
Section J, Fee		
Section K, Total Costs and Fee (I + J)		

Re: DARPA PRE-EMPT

Luke Hamel <hamel@ecohealthalliance.org>

Mon 3/19/2018 1:43 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Hi Tonie,

Screenshots from travel websites (with approximate dates) should be just fine. As for local ground transport, I will check to see how we should move forward with that. I also doubt there will be any car rental.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

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On Mon, Mar 19, 2018 at 2:38 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Arrgh - I didn't realize I needed screen shots. I have just been using expedia to estimate flight costs since I don't have real dates. I guess I'll have to do something different. Also I am guessing at transportation costs. I wasn't anticipating renting a car in China. I assumed we'd be travelling as a group, correct?
>
> On Mon, Mar 19, 2018 at 1:27 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Thanks, Anna.
> >
> > And just a reminder Tonie, that quotes for any travel-related expenses (e.g. airfare, rental car, etc.) must be supported by screenshots, so please forward these and CC our Grants manager, Evelyn Luciano (luciano@ecohealthalliance.org).
> >
> > Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(mobile)
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> I would plan for all your travel to be flying out of Madison. You should have 2 trips to the field sites in Kunming, Yunnan, China (sorry for the typo!), one site visit in Y1 and one deployment visit in Option Year 1. Please remove any x for the Wuhan trip, and enter your needed airfare/lodging. You can use the current federal per diem: $147 for lodging and $115 for meals (for both Wuhan and Kunming). For flights to NYC I would use Laguardia, NYC, though all the NYC airports are equidistance (Newark, Laguardia, or JFK).
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> Let me know if you have any further questions.
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> Best,
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> >
> > On Fri, Mar 16, 2018 at 4:49 PM, Anna Willoughby <willoughby@ecohealthalliance.org> wrote:
> >
> > > Hi Tonie,

Sure thing. Please find attached. I believe this encompasses what we've discussed. We also have in PARC's travel for them to come visit you in Y1 and for some EHA staff (likely Jon Epstein and a Modeling person) to visit Madison once a year.

Please let me know if you have any questions or edits.

Have a great weekend,
Anna

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> > Thanks,
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> > >
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> > >
> > > **From:** Anna Willoughby [mailto:willoughby@ecohealthalliance.org]
> > > **Sent:** Friday, March 16, 2018 12:12 PM
> > > **To:** Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
> > > **Cc:** William B. Karesh <karesh@ecohealthalliance.org>; Peter Daszak <daszak@ecohealthalliance.org>; trocke@usgs.gov; Luke Hamel <hamel@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Fuchs <amanda.andre@ecohealthalliance.org>; Paul, Kateri <Kateri.Paul@parc.com> <Kateri.Paul@parc.com>
> > > **Subject:** Re: DARPA PRE-EMPT
> > >
> > > Thanks for these details, Jerome. Attached are my notes from the call. Action items include:

- Jerome to send more detailed scope of work with paragraphs and revised budget by early next week

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> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company

From: Unidad, Jerome <Jerome.Unidad@parc.com>
Sent: Friday, March 16, 2018 11:52 AM
To: 'William B. Karesh' <karesh@ecohealthalliance.org>; 'Peter Daszak' <daszak@ecohealthalliance.org>
Cc: 'Luke Hamel' <hamel@ecohealthalliance.org>; 'Anna Willoughby' <willoughby@ecohealthalliance.org>; 'Alison Andre' <andre@ecohealthalliance.org>; 'Amanda Andre' <amanda.andre@ecohealthalliance.org>; 'Rocke, Tonie' <trocke@usgs.gov>; Paul, Kateri <Kateri.Paul@parc.com> <Kateri.Paul@parc.com>
Subject: RE: DARPA PRE-EMPT

Peter and team,

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1. Request for Proposal that we can respond to with what they need for their package to DARPA
2. Start date of the proposed effort
3. Contract or a Grant/Other Transaction

Once we have finalized the scope of work and the budget, Kateri will be in touch for these other aspects. Her contact information can be found below.

Kateri E. Paul

Capture Manager, Public Sector

Global Business Development

Palo Alto Research Center (PARC)

3333 Coyote Hill Road

Palo Alto, CA 94304

Kateri.Paul@parc.com

650-812-4821 (desk)

617-596-2023 (mobile)

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Unidad, Jerome <Jerome.Unidad@parc.com>
Sent: Thursday, March 15, 2018 3:33 PM
To: 'Rocke, Tonie' <trocke@usgs.gov>; William B. Karesh <karesh@ecohealthalliance.org>; Johnson, David <David.Johnson@parc.com> <David.Johnson@parc.com>
Cc: Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: RE: DARPA PRE-EMPT

Dear all,

10AM-11AM PST (12PM-1PM CT, 1PM-2PM ET) should work for us. I shall setup a WebEx meeting for this, given the number of participants.

Let me know if this timeslot will work.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 2:39 PM
To: William B. Karesh <karesh@ecohealthalliance.org>
Cc: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>; Peter

Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I assume that is ET? -T

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>
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>
> BK
>
> **William B. Karesh, D.V.M**
>
> *Executive Vice President for Health and Policy*
>
> EcoHealth Alliance
>
> 460 West 34th Street - 17th Floor
>
> New York, NY 10001 USA
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Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

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On Thu, Mar 15, 2018 at 3:42 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

> Actually – can we do a phone call – I'll be driving. 5.15pm would be perfect (NYC time), Today Thursday.
>
> Is that possible?
>
> Our call in line is: 1-719-785-9461

Passcode: 9784#

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

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www.ecohealthalliance.org

@PeterDaszak

@EcoHealthNYC

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From: Jerome.Unidad@parc.com [mailto:Jerome.Unidad@parc.com]
Sent: Thursday, March 15, 2018 4:23 PM
To: trocke@usgs.gov
Cc: William B. Karesh; Peter Daszak; Luke Hamel
Subject: RE: DARPA PRE-EMPT

I can setup a WebEx quickly if we will have multiple parties.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 1:22 PM
To: Unidad, Jerome <Jerome.Unidad@parc.com>
<Jerome.Unidad@parc.com>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Daszak Peter <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I'm available as well. Billy, do you have a call in number? -Tonie

On Thu, Mar 15, 2018 at 3:20 PM, <Jerome.Unidad@parc.com> wrote:

> Dear all,
>
> Sorry for the late response – yes, I will be available for a phone call now. Up to 2PM.
>
> Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: William B. Karesh [mailto:karesh@ecohealthalliance.org]
Sent: Thursday, March 15, 2018 12:49 PM
To: Unidad, Jerome <Jerome.Unidad@parc.com>
<Jerome.Unidad@parc.com>
Cc: Rocke, Tonie <trocke@usgs.gov>; Peter Daszak
<daszak@ecohealthalliance.org>; Luke Hamel
<hamel@ecohealthalliance.org>
Subject: DARPA PRE-EMPT

Dear Dr. Unidad,

Thanks for your quick responses to Dr. Rocke. Would you be available for a short call with Dr. Daszak, Dr. Rocke and me this afternoon or Friday.

We're on tight timeline so we thought a phone call might be save quite a bit of time.

Thanks in advance,

Billy

William B. Karesh, D.V.M

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Tonie E. Rocke

USGS National Wildlife Health Center

6006 Schroeder Rd.

Madison, WI 53711

608-270-2451

trocke@usgs.gov

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Re: DARPA PRE-EMPT

Anna Willoughby <willoughby@ecohealthalliance.org>

Mon 3/19/2018 1:54 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Luke Hamel <hamel@ecohealthalliance.org>

Hi Tonie,

Yes, please add/edit as you feel necessary. You make a good point for the Wuhan trip, please modify to 4 days.

Anna

On Mon, Mar 19, 2018 at 2:35 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> OK thanks for the clarification. Is the trip to Wuhan really only 3 days? Seems like a fast trip considering it takes 24 hours to fly there and back. Also, I am adding trips to local field sites in my area. I am just going to add those at the bottom. Hopefully that is not too confusing. Thanks - Tonie
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> > >
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Executive Vice President for Health and Policy

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Peter Daszak

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@PeterDaszak

@EcoHealthNYC

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Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: William B. Karesh [mailto:karesh@ecohealthalliance.org]
Sent: Thursday, March 15, 2018 12:49 PM
To: Unidad, Jerome <Jerome.Unidad@parc.com>
<Jerome.Unidad@parc.com>
Cc: Rocke, Tonie <trocke@usgs.gov>; Peter Daszak
<daszak@ecohealthalliance.org>; Luke Hamel
<hamel@ecohealthalliance.org>
Subject: DARPA PRE-EMPT

Dear Dr. Unidad,

Thanks for your quick responses to Dr. Rocke. Would you be available for a short call with Dr. Daszak, Dr. Rocke and me this afternoon or Friday.

We're on tight timeline so we thought a phone call might be save quite a bit of time.

Thanks in advance,

Billy

William B. Karesh, D.V.M

Executive Vice President for Health and Policy

EcoHealth Alliance

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New York, NY 10001 USA

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+1.212.380.4465 (fax)

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President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

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--

Tonie E. Rocke

USGS National Wildlife Health Center

6006 Schroeder Rd.

Madison, WI 53711

608-270-2451

trocke@usgs.gov

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Anna Willoughby

Research Assistant

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Re: NWHC budget documents

Luke Hamel <hamel@ecohealthalliance.org>

Tue 3/20/2018 9:07 AM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Anna Willoughby <willoughby@ecohealthalliance.org>; Daszak Peter <daszak@ecohealthalliance.org>

Excellent. Thank you, Tonie! My apologies for the delay in regards to the support letter. I will work to get that to you as soon as possible today.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
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On Tue, Mar 20, 2018 at 9:51 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hello Luke and Anna: Attached are my completed budget, justification, and screen shots for airfare for DARPA. Please let me know what else you need from me. (Note the airfare to Wuhan was totally ridiculous ~7K but if I have to provide screenshots, then I have to use the government travel agency website; we have ways to reduce that cost once we actually make the reservation). None of our supplies cost >5K per item, so I just used lump sum method. I am still waiting on the statement you need signed (NWHC director is in the office today, so it would be helpful to get that as soon as possible) and to update my narrative from Peter. Hope all is going well on your end. Best -Tonie
>
> --
> Tonie E. Rocke
> USGS National Wildlife Health Center
> 6006 Schroeder Rd.
> Madison, WI 53711

608-270-2451
trocke@usgs.gov

Re: [RESCHEDULED] Call to discuss PREEMPT proposal

Luke Hamel <hamel@ecohealthalliance.org>

Tue 3/20/2018 10:04 AM

To: Rocke, Tonie E <trocke@usgs.gov>

Wonderful. Thank you so much, Tonie.

Luke Hamel
Program Assistant

EcoHealth Alliance
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On Tue, Mar 20, 2018 at 10:32 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Yes that would work fine. -Tonie
>
> On Tue, Mar 20, 2018 at 9:20 AM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Would you be available for a call this Wednesday, around 6 PM (ET)?
> >
> > **Luke Hamel**
> > *Program Assistant*
> >
> > EcoHealth Alliance
> > 460 West 34th Street – 17th floor
> > New York, NY 10001
> >
> > (b) (6) (direct)
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ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Tue, Mar 20, 2018 at 10:12 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

No actually I return on Sunday the 25th and could be available on the 26th if necessary. -Tonie

On Tue, Mar 20, 2018 at 9:09 AM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

Thank you for letting me know, Tonie. May I ask when you'll be returning from your trip? Presumably, not before the 27th of March.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
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On Tue, Mar 20, 2018 at 10:06 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

HI Luke: I will be on my way to Mexico on 3/22 so that doesn't work for -sorry! -Tonie

On Tue, Mar 20, 2018 at 9:03 AM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

Hi Tonie,

I apologize for the short notice, but we will have to **reschedule the PREEMPT call to Thursday of this week (3/22)**. This will allow us more time to clean up the proposal, and ensure that our discussion on Thursday is as fruitful as possible.

Please use this link to select the time(s) you are on available on Thu. (3/22) to discuss the proposal. We expect to send you a completed proposal draft by Wednesday night (3/21).

Best,

Luke Hamel
Program Assistant

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trocke@usgs.gov

Re: NWHC budget documents

Anna Willoughby <willoughby@ecohealthalliance.org>

Tue 3/20/2018 10:23 AM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Daszak Peter <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>

Hi Tonie,

Thanks for this information. I have some follow-up questions.

- We should go ahead and use the ~7K airfare for China to can match our quote screenshots. These funds could be redistributed later to other travel as needed.
- We will need materials to be further broken down by item, I am attaching an example. Even for animal per diem, would be nice to have specifics (cage maintenance, etc) We do not need quotes for these items as they are <$5,000 and not considered equipment.
- Do you want Dr. Abbott to attend the Annual Meetings? There seems room in your budget to add this and is standard for subcontract key personnel.
- Could you please provide a small bio for you and Dr. Abbott, here is an example from Peter:

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based research organization focused on emerging zoonotic diseases. His >300 scientific papers include the first global map of EID hotspots *(31, 32)*, estimates of unknown viral diversity *(33)*, predictive models of virus-host relationships *(7)*, and evidence of the bat origin of SARS-CoV *(34, 35)* and other emerging viruses *(36,37,38,39)*. He is Chair of the NASEM Forum on Microbial Threats, and is a member of the Executive Committee and the EHA institutional lead for the $130 million USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr. Daszak won the 2000 CSIRO medal for collaborative research.

We will save any other questions for the phone call with Peter this week. Please let me know if you have any further questions.

Best,
Anna

On Tue, Mar 20, 2018 at 10:07 AM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Excellent. Thank you, Tonie! My apologies for the delay in regards to the support letter. I will work to get that to you as soon as possible today.
>
> Best,
>
> **Luke Hamel**
> *Program Assistant*

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On Tue, Mar 20, 2018 at 9:51 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

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MATE

Item	Manufacturer	Number/Descr	Unit Price
Restriction Enzymes small tubes	NE BIO LABS	R0580S	$72.00
Restriction Enzymes large tubes	NE BIO LABS	R0580L	$292.00
SuperScript™ III Reverse Transcriptase	FISHER	18-080-051	$460.00
T4 DNA Polymerase - 750 units	NE BIO LABS	M0203L	$268.00
Antarctic DNA Phosphatase - 1000 units	NE BIO LABS	M0289S	$68.00
T4 DNA Ligase	NE BIO LABS	M0202L	$256.00
GLOVES PF NITRILE SM (100/pk 10 pk/c	FISHER	19-130-1597E	$249.48
GLOVES PF NITRILE MED (100/pk 10 pk	FISHER	19-130-1597C	$249.48
GLOVES PF NITRILE LG (100/pk 10 pk/c	FISHER	19-130-1597D	$249.48
GLOVES PF NITRILE XL (100/pk 10 pk/c	FISHER	19-130-1597E	$249.48
DMEM with L-Glutamine, 4.5g/L Glucose a	FISHER	MT10013CV	$141.20
Animal per diem for breeder cages	artment of Compartive	hot wash mic	4.2
Animal per diem for experimental cages	artment of Compartive	hot wash mic	8.4

RIALS/EQUIPMENT

Quantity	Total Price	Contract Period
8	$576	Annually, all years
5	$1,460	Annually, all years
2	$920	Annually, all years
2	$536	Annually, all years
3	$204	Annually, all years
2	$512	Annually, all years
3	$748	Annually, all years
2	$499	Annually, all years
2	$499	Annually, all years
2	$499	Annually, all years
1	$141	Annually, all years
365	$1,533	Annually, all years
357	$2,999	Annually, all years

Additional Information
Current price on neb.com
Current price on neb.com
Current price on fishersci.com
Current price on neb.com
Current price on neb.com
Current price on neb.com
Current price on fishersci.com
Current price on fishersci.com
Current price on fishersci.com
Current price on fishersci.com
Current price on fishersci.com
Current UNC DCM rates $0.6 a day per cage for 365 days
Current UNC DCM rates $0.6 a day per cage for 357 days

Re: NWHC budget documents

Anna Willoughby <willoughby@ecohealthalliance.org>

Tue 3/20/2018 11:29 AM

To: Rocke, Tonie E <trocke@usgs.gov>

Yes, the Y2 all partner annual meeting is planned for Wuhan, China. All other years the all-partner annual meeting (1, OY1 and OY0.5) is planned for New York. Thanks for your hard work on this.

Best,
Anna

On Tue, Mar 20, 2018 at 12:21 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Anna: OK, I'm revising again, but I noticed you did not include a Y2 meeting in New York. Is that correct? -Tonie
>
> On Tue, Mar 20, 2018 at 10:23 AM, Anna Willoughby <willoughby@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > Thanks for this information. I have some follow-up questions.
> >
> > - We should go ahead and use the ~7K airfare for China to can match our quote screenshots. These funds could be redistributed later to other travel as needed.
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> > We will save any other questions for the phone call with Peter this week. Please let me know if you have any further questions.
> >
> > Best,
> > Anna

On Tue, Mar 20, 2018 at 10:07 AM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Excellent. Thank you, Tonie! My apologies for the delay in regards to the support letter. I will work to get that to you as soon as possible today.
>
> Best,
>
> **Luke Hamel**
> *Program Assistant*
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> > Madison, WI 53711
> > 608-270-2451
> > trocke@usgs.gov

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Anna Willoughby

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Support letter (PREEMPT)

Luke Hamel <hamel@ecohealthalliance.org>

Tue 3/20/2018 12:26 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Jonathon Musser <musser@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>

Hi Tonie,

Please see the required **collaborator support letter (attached) and comments within.** Thank you for your patience and please let me know if you have any questions.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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20 March 2018

Commented [EA1]: Please insert official letterhead at top of document

Dr. Peter Daszak
President
EcoHealth Alliance
460 West 34th Street
New York, NY 10001

Dear Dr. Daszak:

In regards to the DARPA BAA, PREventing EMerging Pathogenic Threats (PREEMPT), Ref. #: HR001118S0017-PREEMPT-PA-001, PROJECT DEFUSE, the National Wildlife Health Center (NWHC) will be pleased to collaborate with EcoHealth Alliance (EHA) in the implementation of the DEFUSE project should the team be chosen by DARPA to conduct the work.

In our discussions, we have agreed to participate in activities that aim to defuse the potential for spillover and emergence of novel bat-origin high-impact SARS-related coronaviruses from bats to people. Our assistance would include developing and implementing a delivery method for the immune boosting and priming molecules that serve to defuse the potential of disease spillover.

I would also like to confirm that the NWHC has the statutory authority to propose to Government solicitations, such as PREEMPT (please see documentation below). NWHC is a world leader in the development of oral vaccines and delivery methods to manage disease in bats and other free-ranging wildlife. For this reason, and given the 15+ years of collaborative research between the NWHC and EHA, I believe that the NWHC is uniquely capable of addressing the technical challenges listed under PREEMPT.

Furthermore, the NWHC to the best of my knowledge, does not have any conflict of interest with EcoHealth Alliance, nor its collaborators on this project.

On behalf of the NWHC, please list us as a partner in your DEFUSE project proposal. I look forward to working on DEFUSE with EcoHealth Alliance and its partners in this critically important endeavor.

Sincerely,

Commented [2]:
Tonie,

Please note the following information from the BAA (pp. 18-19). **After reviewing the information and link below, please edit the language within this document to correctly describe NWHC's authority/eligibility as it relates to this project. If it's necessary to attach additional documentation, please do so.

"Authority and Eligibility -
At the present time, DARPA does not consider 15 U.S.C. § 3710a to be sufficient legal authority to show eligibility. While 10 U.S.C.§ 2539b may be the appropriate statutory starting point for some entities, specific supporting regulatory guidance, together with evidence of agency approval, will still be required to fully establish eligibility. DARPA will consider FFRDC and Government entity eligibility submissions on a case-by-case basis; however, the burden to prove eligibility for all team members rests solely with the proposer." See link:
https://www.nsf.gov/statistics/ffrdclist/

Commented [3]: Tonie, please include director's signature + printed name here

Identification of pricing assumptions (PREEMPT)

Luke Hamel <hamel@ecohealthalliance.org>

Tue 3/20/2018 3:59 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Hi Tonie,

Please see below, an additional item that we will need to address. Please forward this text to whomever you have been speaking with at NWHC regarding financial guidance for the subaward. They should have a good idea of what this entails for your institution, and this is information that, if relevant, we will need to include within your budget justification.

Taken from p. 27 of the PREEMPT BAA:

"(7) Identification of pricing assumptions of which may require incorporation into the resulting award instrument (e.g., use of Government Furnished Property/Facilities/Information, access to Government Subject Matter Expert/s, etc.)"

Please let me know if you have any questions regarding this matter.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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www.ecohealthalliance.org

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Re: Support letter (PREEMPT)

Luke Hamel <hamel@ecohealthalliance.org>

Tue 3/20/2018 4:01 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Richgels, Katherine L <krichgels@usgs.gov>

Excellent. Thank you very much, Tonie. Please don't hesitate to reach out if any additional concerns arise.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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On Tue, Mar 20, 2018 at 4:59 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Luke: OK, we are working on it. We have gotten money from DOD SERDP, so I don' t believe there should be an issue, but it might take awhile to figure out the right language. Katie Richgels (my boss) is taking care of this and will send it on to you when it is finished and signed. Best -Tonie
>
> On Tue, Mar 20, 2018 at 2:05 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > I apologize that the link did not provide helpful guidance. I've spoken with fellow staff at EHA and they recommend speaking with either: (1) The director or other representative of NWHC's grant department (if your institution has such a department), or (2) Someone in NWHC's Operations or Finance department.
> >
> > My colleague does not think DARPA will give additional guidance regarding this matter. Please let me know if this information helps.
> >
> > Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Tue, Mar 20, 2018 at 1:52 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Luke: I have no idea how to respond about the eligibility. Is there someone we can call at DARPA. The link you provided was no help at all and in fact includes no DOI agencies. -Tonie
>
> On Tue, Mar 20, 2018 at 12:26 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > Please see the required **collaborator support letter (attached) and comments within.** Thank you for your patience and please let me know if you have any questions.
> >
> > Best,
> >
> > **Luke Hamel**
> > *Program Assistant*
> >
> > EcoHealth Alliance
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> >
> > ***EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.***

> > > --
> > > Tonie E. Rocke
> > > USGS National Wildlife Health Center
> > > 6006 Schroeder Rd.
> > > Madison, WI 53711
> > > 608-270-2451
> > > trocke@usgs.gov

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
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608-270-2451
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Re: DARPA PRE-EMPT

Anna Willoughby <willoughby@ecohealthalliance.org>

Tue 3/20/2018 4:15 PM

To: Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Peter Daszak <daszak@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>; Luke Hamel <hamel@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Fuchs <amanda.andre@ecohealthalliance.org>; Kateri.Paul@parc.com <Kateri.Paul@parc.com>

Dear all,

Please find the NWHC section of the proposal. Peter is currently working on an updated draft, but this should be sufficient for composing your budget and particular task. Please let me know if you have any questions. If needed, I am also attaching the original BAA for the program, with PARC's focus being TA2.

When can we expect the revised budget and scope of work?

Best,
Anna

On Fri, Mar 16, 2018 at 3:12 PM, Anna Willoughby <willoughby@ecohealthalliance.org> wrote:

> Thanks for these details, Jerome. Attached are my notes from the call. Action items include:
>
> *- Jerome to send more detailed scope of work with paragraphs and revised budget by early next week*
> *- EHA will send PARC the NWHC section of the proposal on Monday*
> *- EHA will send the format of letter of support for PARC*
> *- EHA to follow up with Kateri with requested information*
>
> For your question on collaborating with other institutes, it is likely that all organizations involved may have insight into the aerosol-bat interaction. I believe this topic would be covered during the Annual Meeting between all partners, as well as during relevant cross-partner trips, in addition to monthly conference calls.
>
> Please let us know if you have further questions.
>
> Best,
> Anna
>
> On Fri, Mar 16, 2018 at 2:57 PM, <Jerome.Unidad@parc.com> wrote:

An additional point for Peter, Tonie (and everyone),

For the spray technology, refinement of the details with respect to aerosol-bat interaction (i.e. the preliminary field testing to see how bats react to the aerosol) and eventual field-deployment in China, will the technical lead for coordinating this segment of the project be USGS – National Wildlife Center? Or should we also expect to work/coordinate with other institutes who would give feedback and insights on how this works?

Thanks. This is just for our information.

Best,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Unidad, Jerome <Jerome.Unidad@parc.com>
Sent: Friday, March 16, 2018 11:52 AM
To: 'William B. Karesh' <karesh@ecohealthalliance.org>; 'Peter Daszak' <daszak@ecohealthalliance.org>
Cc: 'Luke Hamel' <hamel@ecohealthalliance.org>; 'Anna Willoughby' <willoughby@ecohealthalliance.org>; 'Alison Andre' <andre@ecohealthalliance.org>; 'Amanda Andre' <amanda.andre@ecohealthalliance.org>; 'Rocke, Tonie' <trocke@usgs.gov>; Paul, Kateri <Kateri.Paul@parc.com> <Kateri.Paul@parc.com>
Subject: RE: DARPA PRE-EMPT

Peter and team,

I'm currently working on putting together a revised budget and equivalent statement of work (tasks breakdown) for PARC's involvement with the project. You can expect this about early next week – approximately Monday. Officially, for the submission, our capture manager, Kateri Paul, who takes care of the other things would need the following things from your equivalent to facilitate our parts of the submission.

1. Request for Proposal that we can respond to with what they need for their package to DARPA
2. Start date of the proposed effort

3. Contract or a Grant/Other Transaction

Once we have finalized the scope of work and the budget, Kateri will be in touch for these other aspects. Her contact information can be found below.

Kateri E. Paul

Capture Manager, Public Sector

Global Business Development

Palo Alto Research Center (PARC)

3333 Coyote Hill Road

Palo Alto, CA 94304

Kateri.Paul@parc.com

650-812-4821 (desk)

617-596-2023 (mobile)

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Unidad, Jerome <Jerome.Unidad@parc.com>
Sent: Thursday, March 15, 2018 3:33 PM
To: 'Rocke, Tonie' <trocke@usgs.gov>; William B. Karesh <karesh@ecohealthalliance.org>; Johnson, David <David.Johnson@parc.com> <David.Johnson@parc.com>
Cc: Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: RE: DARPA PRE-EMPT

Dear all,

10AM-11AM PST (12PM-1PM CT, 1PM-2PM ET) should work for us. I shall setup a WebEx meeting for this, given the number of participants.

Let me know if this timeslot will work.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 2:39 PM
To: William B. Karesh <karesh@ecohealthalliance.org>
Cc: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I assume that is ET? -T

On Thu, Mar 15, 2018 at 4:14 PM, William B. Karesh <karesh@ecohealthalliance.org> wrote:

> Tonie and Jerome,
>
> We would still like to speak. Anytime on Friday between 11:00 AM and 2:00 PM would be great.
>
> BK
>
> **William B. Karesh, D.V.M**

Executive Vice President for Health and Policy

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President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

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On Mar 15, 2018, at 4:55 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

Hi all: Since we didn't hear back from EcoHealth Alliance, Jerome and I went ahead with a short call we had been planning anyway regarding some technical details. I told him our concerns about the proposed budget and we think we have a pretty good plan to reduce the scope of work to the funds we have available. PARC is very unique in developing this technology and their technology fits very well with other work I am doing, so we both feel pretty confident we can work something out. If you still wish to have a discussion

among all of us, we can schedule that for tomorrow, as I believe Jerome had another meeting to run off to for the rest of the day. I'm available the rest of the day if you wish to chat about this in person. Best -Tonie

On Thu, Mar 15, 2018 at 3:42 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

> Actually – can we do a phone call – I'll be driving. 5.15pm would be perfect (NYC time), Today Thursday.
>
> Is that possible?
>
> Our call in line is: 1-719-785-9461
>
> Passcode: 9784#
>
> Cheers,
>
> Peter
>
> **Peter Daszak**
>
> *President*
>
> EcoHealth Alliance
>
> 460 West 34th Street – 17th Floor
>
> New York, NY 10001

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@PeterDaszak

@EcoHealthNYC

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From: Jerome.Unidad@parc.com [mailto:Jerome.Unidad@parc.com]
Sent: Thursday, March 15, 2018 4:23 PM
To: trocke@usgs.gov
Cc: William B. Karesh; Peter Daszak; Luke Hamel
Subject: RE: DARPA PRE-EMPT

I can setup a WebEx quickly if we will have multiple parties.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 1:22 PM
To: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Daszak Peter <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I'm available as well. Billy, do you have a call in number? -Tonie

On Thu, Mar 15, 2018 at 3:20 PM, <Jerome.Unidad@parc.com> wrote:

> Dear all,
>
> Sorry for the late response – yes, I will be available for a phone call now. Up to 2PM.
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company
>
> ---
>
> **From:** William B. Karesh [mailto:karesh@ecohealthalliance.org]
> **Sent:** Thursday, March 15, 2018 12:49 PM
> **To:** Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
> **Cc:** Rocke, Tonie <trocke@usgs.gov>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
> **Subject:** DARPA PRE-EMPT
>
> Dear Dr. Unidad,
>
> Thanks for your quick responses to Dr. Rocke. Would you be available for a short call with Dr. Daszak, Dr. Rocke and me this afternoon or Friday.
>
> We're on tight timeline so we thought a phone call might be save quite a bit of time.
>
> Thanks in advance,
>
> Billy

William B. Karesh, D.V.M

Executive Vice President for Health and Policy

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EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

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--

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--

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--

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--

Anna Willoughby

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--

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Research Assistant

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Task 7: Develop and assess delivery methods to bats for immune boosting and priming molecules

Description and execution: While work is proceeding to identify and optimize immunomodulating agents to manage SARS-Coronaviruses, we will concurrently develop and test mediums, routes, and methods of delivery to large colonies of bats. Several different approaches or combinations of approaches will be assessed to determine the most feasible and simplest method of delivery that achieves high uptake by bats, is safe for humans as well as target and non-target species, and minimizes disturbance to the colony. Sticky edible gels or pastes that bats groom from themselves and each other have been used previously to deliver pharmaceuticals to bats orally and are currently being tested as a medium for delivery of vaccines against rabies and other diseases in wild bats (see preliminary data). These may also be useful for delivering immune modulators and recombinant SARSr-CoV spike proteins to *Rhinolophus* bats, but may need to be combined with viral vectors (like poxvirus or adenovirus) or nanoparticles/nanoemulsions that enhance uptake through mucous membranes or transdermally after topical application.

Poxviruses in particular have been demonstrated to be effective viral vectors for delivering vaccines to wildlife (Slate et al., 2009) Freuling et al., 2013; Rocke et al., 2017). Recent laboratory studies in bats have shown that poxviruses can replicate safely at high levels in bats after oronasal administration (Stading et al., 2016)m and poxvirus vectored vaccines are immunogenic, protecting bats from rabies challenge (Stading et al 2017; see preliminary data). Poxviruses are highly safe, having been tested in a wide variety of wild and domestic animals, they allow for large inserts of foreign DNA, and they have a proven record of success. Poxviruses are good candidates for this project, but we will also consider others.

In addition to viral vectors, we will also consider methods to achieve transcutaneous delivery of the immune boosting proteins without the use of live agents. Recent advances in methods to achieve transdermal or transcutaneous delivery of drugs and vaccines have been reported. (Roberts et al., 2017). However, a major impediment to this route of vaccination is the stratum corneum, the outermost barrier layer of the skin that protects underlying layers from infection and damage. Numerous approaches have relied on mechanical methods to compromise the stratum corneum to allow the drug or vaccine to penetrate into the skin (Roberts et al., 2017). Innovations in nanotechnology show promise in being able to deliver drugs and vaccines into the deeper layers of the skin without the need for damage to the stratum corneum (Mishra et al., 2013), an important consideration. Dendritic cells and Langerhans cells, antigen-presenting cells which reside in the dermis and epidermis, can take up these transdermally delivered proteins and generate an immune response. We are currently testing poly lactic-co-glycolic acid (PLGA) as a nanoparticle to encapsulate rabies glycoprotein as a method of transcutaneous delivery of vaccine to bats. PLGA has been used previously to deliver both toll-like receptor agonists and antigens simultaneously to mice (Ebrahimian, 2017). This and other products (outlined above in Task ?) could potentially be useful with SARSr-CoV glycoproteins. Adjuvants can also be incorporated into nanoemulsions and nanoparticles to amplify the natural immune response to the vaccine antigens (Karande and Mitragotri, 2010). With SARS-CoV spike proteins, the adjuvant Matrix M1

(Isconova, Sweden) has been shown to significantly enhance the immune response in mice (Coleman et al. 2014)

In collaboration with Dr. Baric and others, we will determine the most likely immunomodulating formulations based on the results of TA2, previous animal studies and other available data and then use both laboratory and field studies to assess and optimize delivery vehicles and methods for wild bats. To reduce costs, initial studies will be conducted with locally acquired insectivorous bats (*Eptesicus fuscus*--big brown bats). We have successfully maintained and housed big brown bats and other insectivorous species for several experiments at our facility previously (Stading et al., 2016, 2017). We will treat bats via topical application with various test formulations that include the biomarker Rhodamine B (RB), co-house them with untreated bats, and monitor transfer between bats by collecting hair and whiskers for biomarker analysis. Rhodamine B is detectable within the hair of animals within 24 hours of consumption using a fluorescence microscope, and we have considerable experience using this biomarker for similar studies (see preliminary data).

Once we have confirmed uptake in laboratory studies, we will then assess mass delivery methods in local caves and hibernacula (using biomarker-labeled mediums but without immunomodulatory substances). We will test several different approaches including aerosolization via sprayers that could be used in cave settings and automated sprays triggered by timers and movement detectors at critical cave entry points. Within one week of application, bats will be trapped at the cave entrace using mist nets or Harp traps and hair will be collected to assess the rate of uptake via biomarker analysis. The bats will be released immediately afterward. The procedures will be tested at several different locations as it will likely take some manipulation to determine appropriate dosages for maximum uptake. After we have determined the most optimal approaches for mass delivery, we will then test them on wild bats in our three cave sites in Yunnan Province. Again, biomarker will be used to assess rates of uptake and this data can then be used in modeling studies to help determine the optimal rates of application of immunomodulating agents. Biomarker studies can also be used to assess uptake by non-target species, an important consideration in evaluating safety. Fieldwork will be conducted in collaboration with Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance).

Preliminary Data: Rocke and colleagues have developed oral vaccines and delivery methods to manage disease in free-ranging wildlife for many years, including a sylvatic plague vaccine for prairie dogs (Rocke et al., 2017), and more recently, vaccines against rabies (Stading et al., 2017) and white-nose syndrome for bats (Rocke, unpublished data). In addition to developing, testing and registering vaccines for experimental field use, vaccine delivery methods and uptake by the target species were optimized using biomarker studies prior to deployment; biomarker studies were also used to assess uptake and safety in non-target hosts (Tripp et al., 2015). A similar approach will be used to develop, test and optimize delivery methods to *Rhinolophus* bats in SE Asia.

To manage plague caused by *Yersinia pestis* in prairie dogs, a raccoon poxvirus vectored vaccine expressing plague antigens was incorporated into a peanut-butter flavored bait matrix. Rhodamine B (RB), a biomarker that dyes hair, whiskers and feces and is visible within 24 hours of consumption by animals, was included in the baits in

order to assess uptake by both target and non-target species (Figure 1). When viewed under a UV microscope at a specific wavelength, the biomarker is visible until the hair grows out (approximately 50 days in prairie dogs). Biomarker studies were initially used to assess palatability and acceptance of the bait matrix by wild prairie dogs (Tripp et al., 2014) and also used to assess bait ingestion by non-target rodents (Tripp et al., 2015). After safety was confirmed in non-targets and with the approval of USDA Center for Veterinary Biologics, a large field trial was conducted over a 3-year period that demonstrated vaccine effectiveness in four species of prairie dogs in seven western states (Rocke et al., 2017). Using biomarker analysis, we then assessed site- and individual host-level factors related to bait consumption in prairie dogs to determine those most related to increased bait consumption, including age, weight, and the availability of green vegetation. Identifying the factors that maximize the likelihood of expedient bait uptake by targeted individuals is important for developing strategies to optimize vaccine effectiveness. This will also be important in developing disease management strategies for bats.

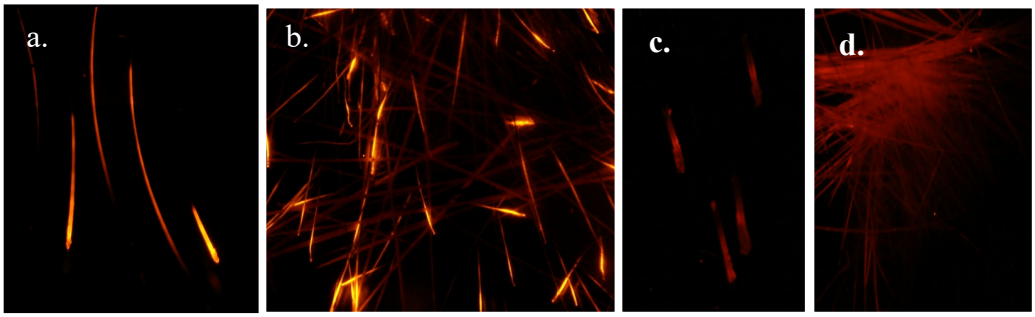

Figure 1. Prairie dog hair and whisker samples viewed under fluorescence microscope (excitation wavelength: 540 nm, emission wavelength: 625 nm) to determine uptake of baits containing Rhodamine B. a) whiskers positive for RB uptake 20 days after bait distribution, b) hair sample positive for RB uptake 16 days after bait distribution, c and d) whiskers and hair negative for RB uptake 20 days after bait distribution (note natural dull fluorescence).

In recent years, our research team has been developing and testing vaccines and delivery methods for use in free-ranging bats. First we tested two commonly used viral vectors, modified vaccinia Ankara (MVA) and raccoon poxvirus (RCN), for their safety and replication in bats using in vivo biophotonic imaging. (Stading et al. 2017). RCN replicated to higher levels in bats than MVA, even via the oral route, and was found to be highly safe for bats (Figure 2). We then used raccoon poxvirus as a viral vector to express a novel rabies glycoprotein (mosaic or MoG) and tested the protective efficacy of this construct in bats after both oronasal and topical administration (Stading et al 2017). Both methods of application were successful, protecting nearly all of the immunized and challenged bats (Figure 3), work is now progressing to develop methods of vaccine delivery to vampire bats, one of the primary reservoirs of rabies for both humans and animals, primarily cattle, in several Latin American countries. We are also using a similar approach to develop vaccines for white-nose syndrome in bats, a devastating disease that has killed millions of insectivorous bats in North America.

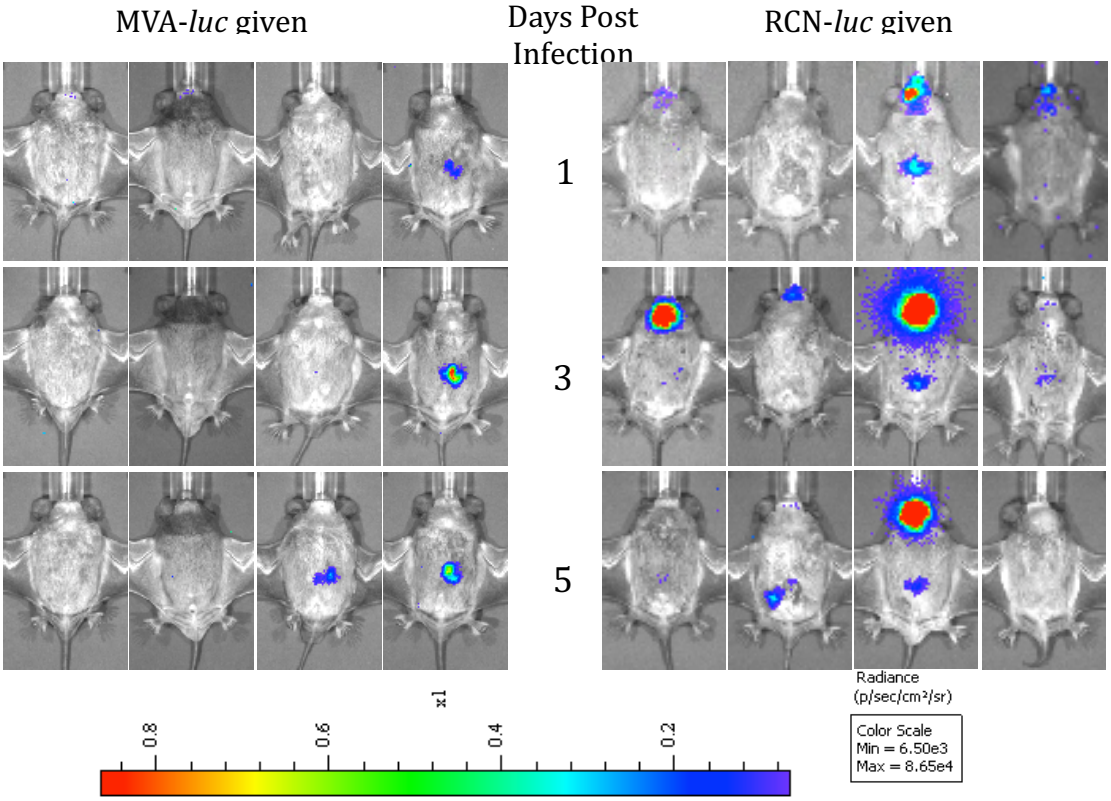

Figure 2. Luminescence, indicative of viral replication of modified vaccinia Ankara (MVA) and raccoon poxvirus RCN) in *Tadarida brasiliensis* on days 1, 3 and 5 post-inoculation via the oronasal route.

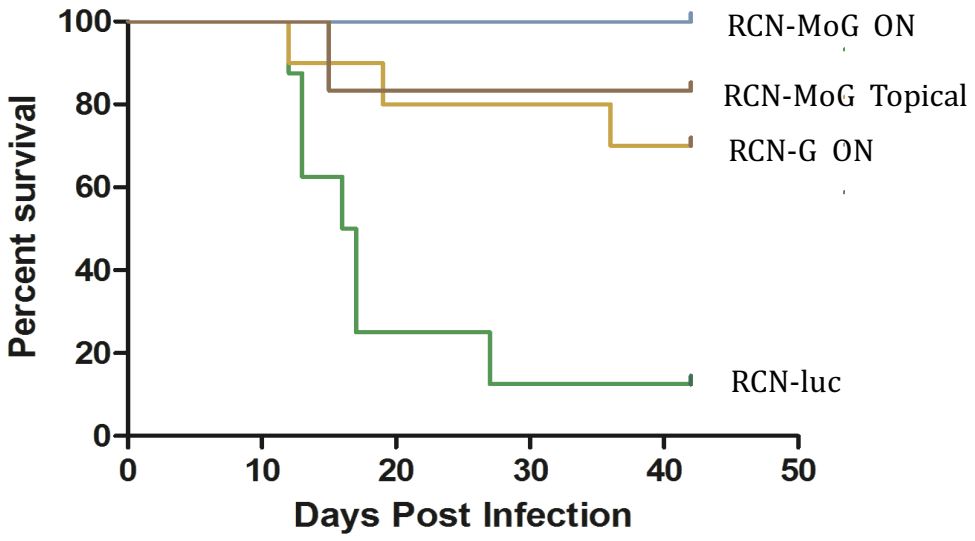

Figure 3. Results of vaccine efficacy and rabies challenge trials in *Epstesicus fuscus* immunized with raccoon poxvirus expressing a mosaic G protein (RCN-MoG) either oronasally (ON) or topically in comparison to RCN expressing typical G protein and RCN expressing luciferase (a negative control).

For bats a different approach is required for vaccine delivery, as in general, they are not attracted to baits. Bats, especially vampire bats, are known to practice self and mutual grooming at a high rate, and this behavior has been exploited to cull vampire bats using poisons like warfarin. The poison is applied topically to a number of bats that are released. When they return to their roost, the poison is transferred to roost-mates by contact and mutual grooming. We are exploiting this same behavior for vaccine application. Preliminary biomarker studies (without vaccine) are being conducted in vampire bats in both Mexico and Peru and also in insectivorous bats in Wisconsin. In a pilot study in Peru, we treated 50 bats from a single cave with RB-labelled glycerin jelly. Based on capture-recapture data, we estimated the population at ~200 bats, so ~25% of bats were initially marked. Upon trapping of this population a few days later, 64 bats were captured, including 19 originally marked bats (Table 1 – could be made into a figure instead). Hair was collected and examined for RB marking under a fluorescence microscope. All treated bats were positive for RB marking in addition to 39% of newly captured bats, indicating a rate of transfer of about 1.3 bats for every bat marked. Additional trials have been conducted, with transfer rates of up to 2.8 bats for every bat treated achieved at least once. These trials are being analyzed to assess factors associated with rates of transfer, e.g. sex and age of initially treated bats, time of day, etc. This data is then being used to model the rate of vaccination and impact on rabies transmission with different rates of application, prior to actual deployment of vaccine in the field.

Table 1. Marking of vampire bats a few days after application of glycerin jelly containing Rhodamine B.

	Number captured	**Positive**	**Negative**	**Inconclusive**	**% positive (w/o inc)**
All bats	64	34	25	5	58
Recaptured marked bats	19	18	0	1	100
New bat captures	45	16	25	4	39

For insectivorous bats, we are trying other approaches. Instead of hand applying the jelly to bats, we applied RB marked glycerin jelly to the entry of bat houses used by little brown bats (*Myotis lucifugus*). The bats became covered as they entered the houses and then consumed the material during self and mutual grooming. One week later, bats were trapped at the houses to determine the rate of uptake. Of 29 bats trapped one week post-application, 59% (17) were positive for biomarker indicating they had eaten the jelly. Thus, with additional optimization, application of vaccine to bat houses or other

structures (small cave entrances) could also be a viable method of delivery. In addition, we are considering different spray applications directly to roosting bats in caves and through motion-sensing sprayers at cave entrances. Whatever the means of application, effective treatment relies on ingestion by bats, and that is easily confirmed with the use of the biomarker, RB.

Organization leading task: USGS National Wildlife Health Center

Progress Metrics: Not sure exactly what format to use here

Deliverable(s):
Medium and methods to deliver immunomodulatory agents to bats.
Data on uptake in insectivorous bats.
Reports, manuscripts, presentations.

Coleman CM, Liu YV, Mu H, Taylor JK, Massare M, Flyer DC, Smith GE, Frieman MB. 2014. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine 32:3169-3174.

Ebrahimian M, Hashemi M, Maleki M, Hashemitabar G, Abnous K, Ramezani M, Haghparast A. 2017. Co-delivery of dual toll-like receptor agaonists and antigen in poly(lactic-co-glycolic) acid/polyethylenimine cationic hybrid nanoparticles promote efficient in vivo immune responses. Front Immunol 8:1077.

Freuling CM, Hampson K, Selhorst T, Schro¨der R, Meslin FX, Mettenleiter TC, Mu¨ller T (2013) The elimination of fox rabies from Europe: determinants of success and lessons for the future. Philosophical Transactions of the Royal Society London B Biological Sciences 368(1623):20120142 (DOI: 10.1098/rstb.2012. 0142)

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Broad Agency Announcement

PREventing EMerging Pathogenic Threats (PREEMPT)

BIOLOGICAL TECHNOLOGIES OFFICE

HR001118S0017

January 19, 2018

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PART I: OVERVIEW INFORMATION

- **Federal Agency Name** – Defense Advanced Research Projects Agency (DARPA), Biological Technologies Office
- **Funding Opportunity Title –** PREventing EMerging Pathogenic Threats
- **Announcement Type –** Initial
- **Funding Opportunity Number –** HR001118S0017
- **Catalog of Federal Domestic Assistance Numbers (CFDA) – 12.910 Research and Technology Development**
- **Dates**
  - Posting Date – January 19, 2018
  - Proposal Abstract Due Date and Time – February 13, 2018 4:00 ET
  - Proposal Due Date and Time – March 27, 2018 4:00 ET
  - BAA Closing Date – March 27, 2018
  - Proposers' Day – January 30, 2018

  https://www.fbo.gov/spg/ODA/DARPA/CMO/DARPA-SN-18-18/listing.html

- **Concise description of the funding opportunity – DARPA is soliciting innovative proposals to develop novel and scalable approaches to preempt viral spillover and transmission from animals or vectors into humans.**

- **Anticipated individual awards - Multiple awards are anticipated.**
- **Types of instruments that may be awarded - Procurement contract, cooperative agreement or other transaction.**
- **Any cost sharing requirements -** Cost sharing may be required under applicable statutory regulations for other transactions for prototype projects awarded under the authority of 10 U.S.C. § 2371b.
- **Agency contact**
  - Points of Contact
    James Gimlett, Ph.D. Program Manager
    Biological Technologies Office

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PART II: FULL TEXT OF ANNOUNCEMENT

1. Funding Opportunity Description

This publication constitutes a Broad Agency Announcement (BAA) as contemplated in Federal Acquisition Regulation (FAR) 6.102(d)(2) and 35.016 and 2 CFR § 200.203. Any resultant award negotiations will follow all pertinent law and regulation, and any negotiations and/or awards for procurement contracts will use procedures under FAR 15.4, Contract Pricing, as specified in the BAA.

DARPA is soliciting innovative proposals for research to develop new tools and models to quantify the likelihood of a virus to jump from an animal host into humans, and to develop and validate new scalable technologies to target potential human-capable viral pathogens in wild reservoirs and/or mosquito vectors to prevent transmission to humans.

1.1. PROGRAM OVERVIEW

Introduction

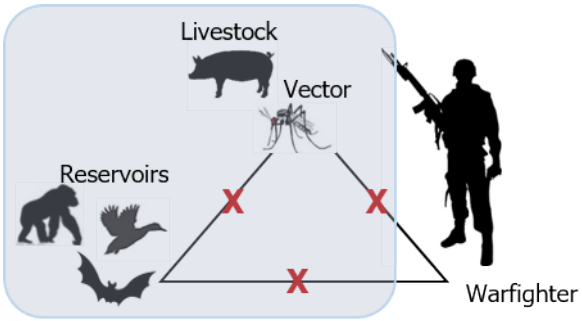
During U.S. international operations, military forces are deployed to remote locations around the globe, often in areas where endemic and emerging diseases are prevalent[1]. Most of these emerging and re-emerging diseases originate in animal reservoirs and then jump into humans. Numerous trends, including the increased interactions between human, animal and insect populations due to increased population densities, globalization, densification of livestock production, and rising human encroachment into animal habitats, have increased the risks of new viral outbreaks in those regions where Department of Defense (DoD) personnel are typically deployed. Often, DoD personnel are among the first responders in outbreak situations. Emerging infectious diseases, for which few medical countermeasures are available, represent a major threat to the warfighter and national security and could have devastating impacts on U.S. public health.

Despite biosurveillance efforts around the globe, new viral outbreaks continue to outpace preparedness efforts and show no signs of abating. During the first three quarters of 2017 outbreaks of avian influenza A (H7N9), Chikungunya, MERS coronavirus, Ebola, Seoul virus, Hepatitis E, Hepatitis A, Yellow Fever, Lassa, and Zika viruses were recorded[2]. While current biosurveillance strategies focus on detection of known pathogens within the human population following an infectious outbreak event, there is a dearth of research and surveillance on sentinel or reservoir animals[3]. Animal-specific viruses that have the potential to infect humans (*namely "*human-capable" pathogens), but have not yet spilled over into human populations, are rarely considered. As a result, infectious agents are detected only *after* an outbreak—that is, after an animal pathogen has adapted to become capable of infecting humans. Consequently, the outbreak response is largely reactive and not initiated until after an epidemic has already begun. The PREEMPT program represents a radical departure from current practice, aiming to target viral

[1] Halliday Jo E.B. et al. (2017). Driving improvements in emerging disease surveillance through locally relevant capacity strengthening. Science.
[2] World Health Organization (2017). http://www.who.int/csr/don/archive/year/2017/en/.
[3] Metcalf, J.E. and Lessler, J. (2017). Opportunities and challenges in modeling emerging infectious diseases. Science.

biothreats within the animal reservoirs where they originate and preempt their entry into human populations *before* an outbreak occurs.

Recently, the scientific community has advanced its understanding of host-pathogen genetics and mechanisms of adaptation across hosts[4,5], developed analytic tools to predict animal hosts of new and potential human-transmissible viruses, and learned how to identify "hot spot" geographic regions where an animal-to-human virus jump is imminent[6,7]. This understanding is empowered by new high-throughput data generation capabilities and sophisticated analytic and computational tools. Together, this new understanding and capability hold great promise for the development of advanced integrated models that can assess and likely provide guidance for action that prevents human virus emergence before the virus gains entry to the human population. The PREEMPT program aims to develop new tools and models to quantify the likelihood of a virus quasispecies (QS) to jump from an animal host into humans. In parallel, PREEMPT seeks to develop and validate new scalable technologies that prevent transmission of viral pathogens in wild reservoirs and/or mosquito vectors to humans or to bridge animals that serve as intermediary hosts prior to virus jump into humans.



Research Objectives

PREEMPT research objectives are structured along two Technical Areas (TAs). Both Technical Areas must be performed in parallel by vertically integrated, interdisciplinary teams. Proposers must present a plan to address both Technical Areas and meet key milestone decision points that occur at the end of year 2.

1) TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region.

2) TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

Technical Area 1 (TA1)

[4] Lloyd-Smith, J.O. (2010). Identifying genetic markers of adaptation for surveillance of viral host jumps. Nature Reviews Microbiology.
[5] Plowright, R.K. et al. (2017). Pathways to zoonotic spillover. Nature Reviews Microbiology.
[6] Olival, K.J. et al. (2017). Host and viral traits predict zoonotic spillover from mammals. Nature.
[7] Han, B.A. et al. (2016). Undiscovered Bat Hosts of Filoviruses. PLoS Negl Trop Dis.

Studies within TA1 must produce and validate models that: (a) quantify the likelihood of a virus to jump into a new animal species and/or humans, (b) identify opportunities for proactive intervention, and (c) determine likely efficacy, scalability, and sustainability of prevention strategies.

Proposers are expected to leverage high-throughput virus screening methods, metagenomics, ecological surveillance, and advanced modeling tools to generate risk models for species jump that will enable near real-time data analysis and identification of potential risks and risk factors. This far-forward biosurveillance system should also identify opportunities for preemptive intervention, assessing likely efficacy, scalability, safety, and sustainability of preemptive strategies to target viral threats in animal reservoirs and/or vectors before they enter the human population.

TA1 Components
Proposers should address, at minimum, the following aspects:

1) Selection of zoonotic or vector-borne viral pathogen(s) (multiple viruses within the same family may be addressed if they share a common animal reservoir and/or vector)
2) Field data collection
3) Multi-species field samples studied in a controlled laboratory setting
4) Data analysis, integration, and model development
5) Real-time data sharing and analysis
6) Model outputs
7) Experimental validation of model predictions in a controlled, environment-simulated laboratory setting

1. Selection of zoonotic or vector-borne viral pathogen

This BAA only will consider proposals focused on zoonotic and/or vector-borne viruses. Microorganisms other than viruses are not responsive to this announcement. A rationale for the viruses selected is required. Virus selection may be based on, but is not limited to, the following factors: high frequency of re-emergence (*e.g.* avian influenza virus), patterns of virus host range or host breadth (predicted zoonotic potential), potential for rapid spread due to vector-mediated transmissibility, severity of disease pathology, and likelihood of pandemic threat.

2. Field data collection

Proposers must identify and justify suitable geographic "hotspots" within which they will collect field data. Proposers must consider <u>all</u> of the following criteria when selecting geographic hot spots for field data collection:

1) Previous evidence of geographic distribution of zoonotic reservoirs and/or vectors for known or unknown human viruses; these maps may be based on epidemiological, phylogenetic, ecological, biogeographic, socio-economic data, or other;
2) Evidence of past species jump events in or near the selected geographic location;

3) Demonstrated capabilities and infrastructure to perform research in the selected geographic region and/or collaboration with an established DoD or Department of Health and Human Services (DHHS) partner (*e.g.*, a Naval Medical Research Unit site, Armed Forces Research Institute of Medical Sciences, or Centers for Disease Control), such that the performer can coordinate far-forward surveillance activities and access local lab and analytics capabilities;
4) Appropriate levels of in-country government approval, cooperation, infrastructure and logistical support where samples will be collected and analyzed; and
5) Rationale for reservoirs/species to be sampled.

Where applicable, proposers must consider seasonal distribution (*e.g.,* wet-dry seasons for mosquito), temporal ecological factors (*e.g.,* time of fruiting for fruit bats), and temporal behavioral traits (*e.g.,* sexual maturation) of zoonotic species for field sampling. Potential geographic areas may include, but are not limited to, endemic regions; those undergoing ecological shifts (thus increasing risk for spillover due to changes in animal-human interactions); those harboring host species with high zoonotic potential that are in proximity to human populations and "bridge" animal hosts (*e.g.,* human-bat-swine ecosystems); and prior sites of spillover events or outbreaks. The selection of geographic areas of common military deployment that also meet the above criteria is strongly encouraged. Proposers should describe feasible approaches to increase the probability of detecting viruses within animal reservoirs and/or vectors—residing in selected geographic areas—that have the potential to become human-capable. Proposers should describe sample collection methods in detail, being sure to include longitudinal sampling frequency. Development of novel and rapid sampling approaches for the real-time continuous screening of emerging or re-emerging pathogens at the human-animal interface is encouraged. Proposers are encouraged to identify field samples that were collected during past outbreak events, or field data already generated, that could be accessed for retrospective analyses. In such cases, proposers should describe how and where the data were collected, and establish quality control methods for data evaluation and use. Although human use research will not be funded by PREEMPT, the use of human samples or data from prior outbreaks obtained through other programs may be included in the research plan as long as samples are appropriately de-identified (see, for example, https://humansubjects.nih.gov/human-specimens-cell-lines-data).

3. Multi-species laboratory testing of field samples

Proposers should discuss protocols to determine and quantify the virus population QS diversity from the vector or reservoir at the time of sample collection (t=0) in a manner that minimizes QS alterations, which commonly result from cell line passaging. Proposers should assess the need for longitudinal collection of samples to understand viral QS temporal dynamics (temporal changes in sequence and fitness landscapes) in field virus populations. The initial viral QS isolated from a field sample (t=0) will be hereon annotated as "QS_0". Proposers must describe *in vitro* and/or *in vivo* experiments to assess jump potential of the QS_0 population to a relevant new host. Experimental approaches to monitor viral species jump may include, but are not limited to: changes in viral population QS during cell line passaging between relevant species; infection of appropriate animal models; infection of natural animal hosts; and controlled, multi-species laboratory ecosystems.

Lab testing should determine the key parameters influencing the probability of a viral QS_0 to jump and adapt to a new host species. Potential parameters across different host animals or vectors may include, but are not limited to:

1) QS diversity profiles
2) Rates of virus infection and amplification
3) Virus incubation period
4) Viremia and viral shedding
5) Transmission bottlenecks
6) Animal host evolutionary and immune pressures

The data generated should enable the development of genotype-to-phenotype maps and the determination of mutation(s) associated with virus jump to a new host.

4. Data analysis, integration, and model development

Proposers should identify the relevant data needed for developing integrated models of risk assessment. Proposers should discuss the development of probabilistic models of virus jump using advanced computational methods and tools, including both model-driven and data-driven approaches. Models should integrate multi-scale and cross-host species data, including but not limited to, field and experimental data (*e.g.,* QS dynamics), ecological data (*e.g.,* demographic, socio-economic, epidemiological, biogeographical, and other metadata), and other relevant data available, especially that generated from past spillover events. Models should consider all factors associated with pathogen emergence and transmission, particularly multi-host immunological landscapes. Models should also capture viral evolutionary trajectories, fitness landscapes in zoonotic and/or vector species, and quantify the transmission dynamics underlying species jump.

5. Real-time data sharing and analysis

The PREEMPT program is expected to generate significant amounts of data, primarily from next generation sequencing (NGS) of viral populations and analysis of host molecular signatures. Proposers should identify methods for near-real-time data sharing and analysis.

6. Model outputs

Proposers should explain how they will develop probabilistic models and machine learning techniques that integrate multi-scale and cross-species data (*e.g.,* molecular signatures, demographic, ecological, socio-economic, epidemiological, weather, climate, and other metadata) to quantify a pathogen's likelihood to cross species barriers and infect humans. Models should capture viral evolutionary trajectories and mutations that govern species jump. Models should quantify transmission dynamics, accounting for the diversity of viral QS. Models should identify key parameters of the pathogen, host species, vector dynamics, and ecological interactions contributing to species jump, and should inform a preemption strategy by identifying optimal pressure points (*e.g.,* jump-enabling mutations, stochastic transmission bottlenecks, and viral amplification requirements) that can be targeted to reduce the likelihood of species jump. For proposals addressing vector-borne viruses, proposers should describe methods to quantify

the likelihood of virus adaptation to a new vector and propose experimental methods to validate these predictions. Proposers should discuss metrics for grading model accuracy, sensitivity, and specificity. Models should be able to receive dynamic biosurveillance inputs and accommodate virus QS changes.

7. Experimental validation of model predictions

Proposers must describe in detail a plan to establish relevant *in vivo*, multi-species experimental approaches to validate model outputs. Experimental testing may closely resemble or recapitulate real-life settings (*e.g.,* climate, phylogenetically adjacent host species, and vector "biting" patterns) to enable the quantification of the probability of spillover and/or transmission events in a controlled manner. Approaches that closely recapitulate real-life ecosystems and natural hosts are strongly encouraged. To improve model accuracy, sensitivity, and specificity, performers must iterate both theoretical and empirical experiments.

TA1 Key Outputs
The key outputs for TA1 must include the following:

1) Integrated models that quantify likelihood of virus jump and can be easily adapted to receive dynamic surveillance and virus data input.
2) Stochastic models quantifying bottlenecks (e.g., transmission, cell entry, and infection rates) and mutational fitness maps (e.g., enabler mutations and their frequency).
3) Identification and assessment of potential preemptive intervention targets to preempt virus jump from the reservoir and/or vector.

Technical Area 2 (TA2)
Studies within this technical area aim to develop deployable and scalable methods to preempt viral jump across species.

TA2 Components
Technical Area 2 aims to develop deployable and scalable methods to preempt viral jump to other species. Proposers must address, at minimum, all of the following aspects:

1) Proof-of-concept preemption approaches;
2) Scalable delivery methods;
3) Analysis of long-term sustainability; and
4) Experimental validation.

1. Proof-of-concept preemption approaches

Proposers should describe how the output of TA1 *in silico* models will guide preventive method design, and how quantitative information of virus-host species barriers and transmission bottlenecks will be used to develop strategies to preempt emergence of human-capable viruses. Models should guide the selection of: host species to be treated (*e.g.*, wild animals, "bridge"

animals, vectors, and livestock); potential molecular targets (*e.g.,* key mutation(s) enabling receptor binding in a new host); targets associated with transmission cycle dynamics (*e.g.,* reduction of viral load within the reservoir and/or vector that would preclude transmission); and other relevant factors identified by the models. Proposers should describe the preemptive methods that address different model outputs. Examples of preemptive approaches include but are not limited to:

1) Specific disruption of jump-capable genes from virus QS in reservoirs and/or vectors using small interfering RNAs or CRISPR/Cas-based targeted deletions.
2) Suppression of virus jump to a new host through antibody-mediated virus neutralization.
3) Suppressed reservoir and/or vector viremia using virus defective interfering particles (DIPs) to outcompete virus replication.
4) Suppressed transmission among animal reservoirs through induced immunity (*e.g.,* vaccinate the animal).
5) Alternative methods informed by experimental and theoretical models. The development of novel preemptive approaches are strongly encouraged.

2. Scalable delivery methods

Proposers must describe scalable approaches to deliver the preemptive therapeutic to achieve animal and/or vector population-level control of the targeted virus, including strategies for reaching less accessible animal reservoirs (*e.g.,* rodents or non-human primates). Approaches that enable host-to-host therapeutic distribution (*i.e.,* do not require individual treatment) that are self-limiting, only activate when the viral pathogen target is present, and/or have a controllable "on/off-switch" are encouraged. Potential scalable methods of inoculation may include, but are not limited to:

1) Self-disseminating treatments or preventives (*e.g.,* transmissible recombinant vaccines, therapeutic interfering particles, or self-spreading antiviral therapies).
2) Bait vaccination or treatment of wild or domestic animals.
3) Spray-based methods.

Approaches that utilize genetic modifications of vectors (*e.g.,* engineered mitochondrial DNA) are acceptable. The proposed method of inoculation must be justified. The proposer must describe strategies for closely controlling preemptive delivery and spread.

3. Analysis of long-term safety and efficacy

Proposers must establish initial methods to assess the long-term safety and efficacy of preemptive approaches (*e.g*., determine the mechanism by which species specificity of a vaccine is maintained, and assess evolutionary stability and ecological safety).

4. Experimental validation

Proposers must describe approaches to validate preemptive methods of choice in controlled experimental models. Multi-species experimental platforms that closely recapitulate real-life ecosystems and use natural hosts are strongly encouraged.

TA2 Key Outputs
The key outputs of TA2 must include the validation of new "block-before-jump" preemption technologies for one of the following:

1) Validate suppression of virus jump from wild animal reservoir to humans and/or an intermediate animal carrier (*e.g.,* domestic livestock).
2) Validate suppression of virus jump or transmission from wild reservoir to vector, vector to a different vector species, and/or from vector to human.

Period of Performance
DARPA anticipates that the PREEMPT program will provide up to three and a half years of funding for research and development to be performed over Phase I (base) and II (option) periods of 24 and 18 months, respectively.

Timeline
PREEMPT spans a 42-month effort with a 24-month Phase I (base) and an 18-month Phase II (option). In general, Phase I should provide early validation of zoonosis risk models, and Phase II should establish efficacy and scalability of zoonosis prevention approaches.

Phase I (Base period)
Phase I efforts aim to develop experimental and mathematical models to quantify the likelihood a virus will jump from one host species to another, identify potential targets for spillover preemption, and develop scalable methods of preemption. During Phase I, performer teams will:

1) Identify the genetic adaptations that enable species jump.
2) Develop mathematical models to quantify the likelihood of species jump based on:
    a. Molecular data (*e.g.,* viral QS data from deep sequencing*)* and
    b. Ecological data *(e.g.,* immune state of the host population before pathogen emergence, species relatedness, etc.).
3) Identify bottlenecks for intervention (*e.g.. transmission, cell entry, viral amplification, infection rate, and other mechanisms associated with viral cross-species compatibility)*.
4) Develop initial scalable platforms that target viruses in reservoirs and/or vectors to prevent viral jump into other animals or humans.

By the end of year 1 (Phase I) performers will be expected to have:
1) Identified signatures of fitness and spillover potential of a pathogen between two species.
2) Quantified the genetic and transmission factors requirements of viral QS to jump to a new host (*e.g.,* develop genotype-to-phenotype maps, identify specific mutations, etc.) using far-forward biosurveillance data from selected high-risk regions.

By the end of year 2 (Phase I) performers will be expected to have:

1) Initially demonstrated that models can quantify the probability of human-capable virus pathogens to jump from one species to another species.
2) Demonstrated proof of concept methods for targeting human-capable virus pathogens in the reservoirs and/or vectors to reduce the probability of virus jump.
3) Provided initial strategies to scale up preemption methods.

Phase II (Option period)
Phase II efforts aim to develop probabilistic models for intra- and inter-species viral amplification and transmission dynamics, integrated models for risk assessment, and experimental validation of new approaches to preempt species jump. During Phase II, performer teams will extend Phase I modeling efforts to:

1) Quantify intra- and inter-species viral amplification dynamics and transmission.
2) Develop integrated models that quantify the probability of a virus QS to jump to bridge animal species or to humans.
3) Experimentally validate scalable methods for their ability to preempt zoonotic spillover.

By the end of year 3.5 (Phase II) performers will be expected to:

1) Demonstrate accuracy of risk assessment and preemption models in a relevant multi-species experimental setting.
2) Demonstrate the ability to suppress viral jump to a new species in controlled experimental settings.

It is recognized that appropriate milestones and metrics may depend upon the type of virus, the reservoir, the mechanisms of species jump, and the proposed preemption methods. Proposers must offer quantitative milestones and metrics (see Tables 1 and 2 below for notional metrics) for their proposed proof-of-principle use case. Proposers must demonstrate relevant research experience in the required technical areas. Proposals involving multiple teams and/or experimental approaches should be structured as unified efforts that address the program Technical Areas in parallel, in an integrated manner.

1.2. PROGRAM METRICS

In order for the Government to evaluate the effectiveness of a proposed solution in achieving the stated program objectives, proposers should note that the Government hereby promulgates the following program metrics that may serve as a guideline for assessing program progress, risk and impact. Although the following program metrics are provided, proposers should note that the Government has identified these goals with the intention of bounding the scope of effort while affording the maximum flexibility, creativity, and innovation in proposing solutions to the stated problem. Proposers should offer more appropriate and specific metrics for their particular use case and technical approach, including intermediate metrics (i.e. every 6 months, or sooner) to help further evaluate progress. Final metrics are to be negotiated at the time of contracting.

Table 1: Notional Milestones, Deliverables, and Program Metrics for TA1

Phase	Milestones and Deliverables	Program Metric
I	Collected field surveillance data: • Virus QS molecular data (*e.g.* from deep sequencing) and metadata from longitudinal samples (*e.g.* obtained from selected high-risk areas (*e.g.* bat cave) and/or from prior outbreak event • Host species immune molecular data	Quantitative measures of: • Longitudinal viral population QS ($QS_{t=0}$, $QS_{t=6\ months}$, $QS_{t=12\ months}$,..) diversity in selected high-risk areas (*e.g.* frequency of mutations, evolutionary trajectories) (**6 months**) • Viral QS diversity in samples obtained from animal, vector, and/or human from prior outbreak event (*e.g.* frequency of species-specific mutations) (**9 months**) • Immune molecular signatures from host reservoir or intermediate reservoir species (**12 months**)
	Multi-species lab test data: • Virus QS genotype-phenotype maps for at least 2 relevant host species	Quantitative measures of: • Cell entry and adaptation across species *in vitro* and/or *in vivo* (*e.g.* QS diversity during passage across species) (**18 months**)
	Initial mathematical models that assess risk of virus jump	Model capability to describe/predict: • Virus QS evolutionary trajectories between 2 relevant species (**9 months**) • Key molecular factors that could be targeted to prevent virus jump *in vitro* and/or *in vivo* (*e.g.* signatures of fitness of a pathogen between two relevant host species) (**18 months**) • Molecular targets for preemption (**24 months**)
	Established testbeds for validation of model predictions	Testbeds mimic natural environment as quantified by performer-defined parameters (**24 months**)
II	Multi-species lab test data • Quantify virus QS transmission factors between two species *in vivo*	Quantitative measures of: • Virus amplification and transmission dynamics (*e.g.* rate of infection vs. viremia, amplification rates, and incubation time) (**30 months**)
	Advanced mathematical models that assess risk of virus jump • Integration of molecular data and virus amplification/transmission dynamics • Integration of host immune evolutionary pressures and virus QS dynamics	Models predict: • Intra- and inter-species transmission dynamics (**36 months**) • Probability of spillover (risk assessment) (**42 months**) • Top 2 targets to reduce probability of transmission between two species to

Phase	Milestones and Deliverables	Program Metric
		inform TA2 (**42 months**)
	Further validation of model prediction in established testbeds	Validated model prediction accuracy in multispecies environment (**42 months**)

Table 2: Notional Milestones, Deliverables, and Program Metrics for TA2

Phase	Milestones and Deliverables	Program Metric
I	Proof-of-concept demonstration of preemptive approach that reduces either the probability of virus jump or the frequency of virus QS variants at high risk for species jump	Quantitative validation of preemptive approach as established by performer (**24 months**) Examples: • Frequency of high-risk mutation within virus QS in reservoir reduced >3X • Virus incubation period in vector extended >3X • Virus amplification rate in reservoir or vector reduced >3X • Viremia in host or vector reduced >5X
II	Demonstrated efficacy of preemption method	Reduced probability of transmission between two species by >5X *in vivo* for top 2 targets (**36 months**)
	Demonstrated scalability of preemption method	Quantitative scalability as established by performer (**42 months**)

Data Sharing

Proposers must ensure all technical data items (including experimental findings, processed data, methods of processing, research reports, and publications) and software (source code and executables) generated from PREEMPT program funding are made available to DARPA. Regularly submitted reports (*e.g.*, monthly or quarterly) should contain all relevant project data, including (but not limited to) raw and analyzed data and any necessary annotations and interpretation. Data and/or samples collected from de-identified human volunteers/patients from previous outbreak events must include associated anonymized metadata (*e.g.,* signs/symptoms, diagnostic test results, interventions, clinical observations, and outcomes). All raw data and metadata should be recorded according to approved experimental standards.

To gain enhanced scientific value from open collaboration in fundamental research, DARPA may seek permission to share some or all program-generated data with the broader research community as open data (including the possibility of accessing, reusing, and redistributing under appropriate licensing terms) to the extent permitted by applicable laws and regulations (*e.g.*, privacy, security, and export control).

DARPA anticipates that a large amount of data will be generated under this program by each performer and that the analyses and validation will be strengthened by compiling and integrating

information across all performers. Performers are strongly encouraged to establish the appropriate agreements to enable collaboration and data sharing. DARPA encourages sharing of pre-existing data, including those generated through funding by other sources, although this is not a requirement of the program.

As feasible, DARPA intends to share data within the PREEMPT performer community to promote program goals. To facilitate sharing and exchange of data items, performers will be required to enter an Associate Contractor Agreement (ACA); an ACA clause will be included in the contract or agreement awarded.

PREEMPT Transition Plan
Proposers must include a PREEMPT Technology Transition Plan. Proposers must indicate the types of partners (*e.g.*, government, private industry, non-profit) they plan to pursue and submit a timeline with incremental milestones toward successful engagement. Proposers should begin transition activities during the early stages of the program (Phase I). Awardees must include DARPA in the development of transition relationships. If the transition plan includes a start-up company, a business development strategy must be included as well. The extent by which the proposed intellectual property (IP) rights will impede the Government's ability to transition the technology will be considered in the proposal evaluation.

1.3. ETHICAL, LEGAL, AND SOCIETAL IMPLICATIONS (ELSI)

DARPA is committed to ensuring that efforts funded under this BAA adhere to ethical and legal regulations currently in place for federally and DoD-funded research. Program developments will be discussed with a panel of expert external advisors with expertise in bioethical and biosafety issues that may emerge as a consequence of advances in biomedical science and technology. Proposers to this BAA should address potential ethical, legal, and societal implications of the proposed technology.

1.4. PROTECTION OF SENSITIVE INFORMATION

PREEMPT is a 6.1 fundamental research program aimed at enhanced biosurveillance and novel approaches to preempt viral pathogens in animal reservoirs from jumping into human populations. DARPA follows current DoD policy for contracted fundamental research. DARPA recognizes, however, that PREEMPT program components aimed at understanding and quantifying mechanisms for viral zoonotic spillover could potentially generate sensitive information that could be misused. Since this is a fundamental research program, the risk of misuse currently cannot be reasonably evaluated. However, proposers are notified that during proposal evaluation and/or program performance, when such a risk reasonably can be evaluated, DARPA may determine that risk of misuse creates exceptional circumstances, compelling reasons, and/or national security reasons under current DoD policy for contracted fundamental research. DARPA therefore expects that proposers to this program understand and will comply with various government guidance regarding potential gain-of-function research of concern (GOFROC)[8] and dual use research of concern (DURC)[9,10,11,12,13]. See https://www.phe.gov/s3/dualuse/Pages/default.aspx for further information.

[8] Gain-of-Function Research (GOFROC) refers to studies with the potential to generate pathogens with pandemic potential exhibiting high transmissibility and high virulence.

DARPA requires that proposals include a Risk Mitigation Plan that will be incorporated into any resulting agreements or contracts and includes the following information:

1) An assessment of potential risks to public health, agriculture, plants, animals, the environment, and national security.
2) Proposed guidelines that the proposer will follow to ensure maximal biosafety and biosecurity during the course of the research.
3) A communication plan that addresses content, timing, and the extent of distribution of potentially sensitive dual-use information. The plan must also address how input from DARPA, other government, and community stakeholders will be taken into account in decisions regarding communication and publication of potentially sensitive dual-use information.

2. Award Information

2.1. GENERAL AWARD INFORMATION

Multiple awards are possible. The amount of resources made available under this BAA will depend on the quality of the proposals received and the availability of funds.

The Government reserves the right to select for negotiation all, some, one, or none of the proposals received in response to this solicitation and to make awards without discussions with proposers. The Government also reserves the right to conduct discussions if it is later determined to be necessary. If warranted, portions of resulting awards may be segregated into pre-priced options. Additionally, DARPA reserves the right to accept proposals in their entirety or to select only portions of proposals for award. In the event that DARPA desires to award only portions of a proposal, negotiations may be opened with that proposer. The Government reserves the right to fund proposals in phases with options for continued work, as applicable. The Government reserves the right to fund a Phase II option based on funding availability, an

[9] Dual Use Research of Concern (DURC) refers to life sciences research that can be reasonably anticipated to provide knowledge, information, products or technology that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security.
[10] Proposed framework for the oversight of dual use life sciences research: strategies for minimizing the potential misuse of research information, National Science Advisory Board for Biosecurity (NSABB). June 2007.
[11] Recommendations for the evaluation and oversight of proposed gain-of-function research by the National Science Advisory Board for Biosecurity (NSABB). May 2016.
[12] Tools for the Identification, Assessment, Management, and Responsible Communication of Dual Use Research of Concern: A Companion Guide to the United States Government Polices for Oversight of Life Sciences Dual Use Research of Concern. NIH. September 2014.
[13] United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern. DURC Policy. March 2012.

assessment of Phase I research results, and a determination that awarding the option is in the best interests of the Government. The Government reserves the right to request any additional, necessary documentation once it makes the award instrument determination. Such additional information may include but is not limited to Representations and Certifications (see Section VI.B.2., "Representations and Certifications"). The Government reserves the right to remove proposers from award consideration should the parties fail to reach agreement on award terms, conditions, and/or cost/price within a reasonable time, and the proposer fails to timely provide requested additional information. Proposals identified for negotiation may result in a procurement contract, grant, cooperative agreement, or other transaction, depending upon the nature of the work proposed, the required degree of interaction between parties, whether or not the research is classified as Fundamental Research, and other factors.

Proposers looking for innovative, commercial-like contractual arrangements are encouraged to consider requesting Other Transactions. To understand the flexibility and options associated with Other Transactions, consult http://www.darpa.mil/work-with-us/contract-management#OtherTransactions.

In all cases, the Government contracting officer shall have sole discretion to select award instrument type, regardless of instrument type proposed, and to negotiate all instrument terms and conditions with selectees. DARPA will apply publication or other restrictions, as necessary, if it determines that the research resulting from the proposed effort will present a high likelihood of disclosing performance characteristics of military systems or manufacturing technologies that are unique and critical to defense. Any award resulting from such a determination will include a requirement for DARPA permission before publishing any information or results on the program. For more information on publication restrictions, see the section below on Fundamental Research.

2.2. FUNDAMENTAL RESEARCH

It is DoD policy that the publication of products of fundamental research will remain unrestricted to the maximum extent possible. National Security Decision Directive (NSDD) 189 defines fundamental research as follows:

> 'Fundamental research' means basic and applied research in science and engineering, the results of which ordinarily are published and shared broadly within the scientific community, as distinguished from proprietary research and from industrial development, design, production, and product utilization, the results of which ordinarily are restricted for proprietary or national security reasons.

As of the date of publication of this BAA, the Government expects that program goals as described herein may be met by proposers intending to perform fundamental research and proposers not intending to perform fundamental research or the proposed research may present a high likelihood of disclosing performance characteristics of military systems or manufacturing technologies that are unique and critical to defense. Based on the nature of the performer and the nature of the work, the Government anticipates that some awards will include restrictions on the resultant research that will require the awardee to seek DARPA permission before publishing any information or results relative to the program.

Proposers should indicate in their proposal whether they believe the scope of the research included in their proposal is fundamental or not. While proposers should clearly explain the intended results of their research, the Government shall have sole discretion to select award instrument type and to negotiate all instrument terms and conditions with selectees. Appropriate clauses will be included in resultant awards for non-fundamental research to prescribe publication requirements and other restrictions, as appropriate. This clause can be found at http://www.darpa.mil/work-with-us/additional-baa.

For certain research projects, it may be possible that although the research being performed by the awardee is restricted research, a subawardee may be conducting fundamental research. In those cases, it is the awardee's responsibility to explain in their proposal why its subawardee's effort is fundamental research

3. Eligibility Information

3.1. ELIGIBLE APPLICANTS

All responsible sources capable of satisfying the Government's needs may submit a proposal that shall be considered by DARPA.

3.1.1. Federally Funded Research and Development Centers (FFRDCs) and Government Entities

FFRDCs

FFRDCs are subject to applicable direct competition limitations and cannot propose to this BAA in any capacity unless they meet the following conditions: (1) FFRDCs must clearly demonstrate that the proposed work is not otherwise available from the private sector. (2) FFRDCs must provide a letter on official letterhead from their sponsoring organization citing the specific authority establishing their eligibility to propose to Government solicitations and compete with industry, and their compliance with the associated FFRDC sponsor agreement's terms and conditions. This information is required for FFRDCs proposing to be awardees or subawardees.

Government Entities

Government Entities (e.g., Government/National laboratories, military educational institutions, etc.) are subject to applicable direct competition limitations. Government entities must clearly demonstrate that the work is not otherwise available from the private sector and provide written documentation citing the specific statutory authority and contractual authority, if relevant, establishing their ability to propose to Government solicitations.

Authority and Eligibility

At the present time, DARPA does not consider 15 U.S.C. § 3710a to be sufficient legal authority to show eligibility. While 10 U.S.C.§ 2539b may be the appropriate statutory starting point for some entities, specific supporting regulatory guidance, together with evidence of agency approval, will still be required to fully establish eligibility. DARPA will consider FFRDC and

Government entity eligibility submissions on a case-by-case basis; however, the burden to prove eligibility for all team members rests solely with the proposer.

3.1.2. Non-U.S. Organizations

Non-U.S. organizations and/or individuals may participate to the extent that such participants comply with any necessary nondisclosure agreements, security regulations, export control laws, and other governing statutes applicable under the circumstances.

3.2. ORGANIZATIONAL CONFLICTS OF INTEREST

FAR 9.5 Requirements

In accordance with FAR 9.5, proposers are required to identify and disclose all facts relevant to potential OCIs involving the proposer's organization and *any* proposed team member (subawardee, consultant). Under this Section, the proposer is responsible for providing this disclosure with each proposal submitted to the BAA. The disclosure must include the proposer's, and as applicable, proposed team member's OCI mitigation plan. The OCI mitigation plan must include a description of the actions the proposer has taken, or intends to take, to prevent the existence of conflicting roles that might bias the proposer's judgment and to prevent the proposer from having unfair competitive advantage. The OCI mitigation plan will specifically discuss the disclosed OCI in the context of each of the OCI limitations outlined in FAR 9.505-1 through FAR 9.505-4.

Agency Supplemental OCI Policy

In addition, DARPA has a supplemental OCI policy that prohibits contractors/performers from concurrently providing Scientific Engineering Technical Assistance (SETA), Advisory and Assistance Services (A&AS) or similar support services and being a technical performer. Therefore, as part of the FAR 9.5 disclosure requirement above, a proposer must affirm whether the proposer or *any* proposed team member (subawardee, consultant) is providing SETA, A&AS, or similar support to any DARPA office(s) under: (a) a current award or subaward; or (b) a past award or subaward that ended within one calendar year prior to the proposal's submission date.

If SETA, A&AS, or similar support is being or was provided to any DARPA office(s), the proposal must include:

- The name of the DARPA office receiving the support;
- The prime contract number;
- Identification of proposed team member (subawardee, consultant) providing the support; and
- An OCI mitigation plan in accordance with FAR 9.5.

Government Procedures

In accordance with FAR 9.503, 9.504 and 9.506, the Government will evaluate OCI mitigation plans to avoid, neutralize or mitigate potential OCI issues before award and to determine whether it is in the Government's interest to grant a waiver. The Government will only evaluate OCI mitigation plans for proposals that are determined selectable under the BAA evaluation criteria and funding availability.

The Government may require proposers to provide additional information to assist the Government in evaluating the proposer's OCI mitigation plan.

If the Government determines that a proposer failed to fully disclose an OCI; or failed to provide the affirmation of DARPA support as described above; or failed to reasonably provide additional information requested by the Government to assist in evaluating the proposer's OCI mitigation plan, the Government may reject the proposal and withdraw it from consideration for award.

3.3. COST SHARING/MATCHING

Cost sharing is not required; however, it will be carefully considered where there is an applicable statutory condition relating to the selected funding instrument. Cost sharing is encouraged where there is a reasonable probability of a potential commercial application related to the proposed research and development effort.

For more information on potential cost sharing requirements for Other Transactions for Prototype, see http://www.darpa.mil/work-with-us/contract-management#OtherTransactions

4. Application and Submission Information

4.1. ADDRESS TO REQUEST APPLICATION PACKAGE

This announcement, any attachments, and any references to external websites herein constitute the total solicitation. If proposers cannot access the referenced material posted in the announcement found at http://www.darpa.mil, contact the administrative contact listed herein.

4.2. CONTENT AND FORM OF APPLICATION SUBMISSION

All submissions, including abstracts and proposals must be written in English with type not smaller than 12 point font. Smaller font may be used for figures, tables, and charts. Copies of all documents submitted must be clearly labeled with the DARPA BAA number, proposer organization, and proposal title/proposal short title.

4.2.1. Proposal Abstract Format

Proposers are strongly encouraged to submit an abstract in advance of a proposal to minimize effort and reduce the potential expense of preparing an out of scope proposal. The abstract is a concise version of the proposal comprising a **maximum of 8 pages** including all figures, tables, charts, and the Executive Summary slide. The (optional) submission letter is not included in the page count. All pages shall be formatted for printing on 8-1/2 by 11-inch paper with font size not smaller than 12 point. Smaller font sizes may be used for figures, tables, and charts.

Submissions must be written in English.

Abstracts must include the following components:

A. Cover Sheet (does not count towards page limit): Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also

include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of the project, and the label "ABSTRACT."

B. Executive Summary Slide: Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Proposers should use the slide template provided as Attachment 1 to the BAA posted at http://www.fbo.gov.

C. Goals and Impact: Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:

1. What is the proposed work attempting to accomplish or do?
2. How is it done today? And what are the limitations?
3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?
4. What are the key technical challenges in your approach and how do you plan to overcome these?
5. Who will care and what will the impact be if you are successful?
6. How much will it cost and how long will it take?

D. Technical Plan: Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.

E. Capabilities: Provide a brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government-furnished materials or data assumed to be available. If desired, include a brief bibliography with links to relevant papers, reports, or resumes of key performers. Do not include more than two resumes as part of the abstract. Resumes count against the abstract page limit.

4.2.2. Proposal Format

All full proposals must be in the format given below. Proposals shall consist of two volumes: 1) **Volume I, Technical and Management Proposal**, and 2) **Volume II, Cost Proposal.** All pages shall be printed on 8-1/2 by 11-inch paper with type not smaller than 12 point. Smaller font may be used for figures, tables and charts. The page limitation for full proposals includes all figures, tables, and charts. Volume I, Technical and Management Proposal, may include an attached bibliography of relevant technical papers or research notes (published and unpublished) which document the technical ideas and approach upon which the proposal is based. Copies of not more than three (3) relevant papers may be included with the submission. The bibliography

and attached papers are not included in the page counts given below. The submission of other supporting materials along with the proposals is strongly discouraged and will not be considered for review. **The maximum page count for Volume 1 is 36 pages.** A submission letter is optional and is not included in the page count. Volume I should include the following components:

NOTE: Non-conforming submissions that do not follow the instructions herein may be rejected without further review.

a. Volume I, Technical and Management Proposal

Section I. Administrative

A. Cover Sheet (LABELED "PROPOSAL: VOLUME I"):

1. BAA number (HR001118S0017);
2. Lead organization submitting proposal (prime contractor);
3. Type of organization, selected from among the following categories: "LARGE BUSINESS," "SMALL DISADVANTAGED BUSINESS," "OTHER SMALL BUSINESS," "HBCU," "MI," "OTHER EDUCATIONAL," OR "OTHER NONPROFIT";
4. Proposer's reference number (if any);
5. Other team members (if applicable) and type of business for each;
6. Proposal title;
7. Technical point of contact (Program Manager or Principle Investigator) to include: salutation, last name, first name, street address, city, state, zip code, telephone, fax, e-mail;
8. Administrative point of contact (Contracting Officer or Grant Officer) to include: salutation, last name, first name, street address, city, state, zip code, telephone, fax, e-mail;
9. Award instrument requested: cost-plus-fixed-free (CPFF), cost-contract—no fee, firm-fixed-price, grant, cooperative agreement, other transaction, or other type (specify);
10. Place(s) and period(s) of performance ;
11. Proposal validity period;
12. Total funds requested from DARPA, and the amount of cost share (if any); AND
13. Date proposal was submitted.

Information on award instruments is available at http://www.darpa.mil/work-with-us/contract-management.

B. Official Transmittal Letter.

Section II. Detailed Proposal Information

A. Executive Summary: Provide a synopsis of the proposed project, including answers to the following questions:

- What is the proposed work attempting to accomplish or do?
- How is it done today, and what are the limitations?
- What is innovative in your approach?
- What are the key technical challenges in your approach and how do you plan to overcome these?
- Who or what will be affected and what will be the impact if the work is successful?
- How much will it cost, and how long will it take?

B. Executive Summary Slide: Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Proposers should use the slide template provided as **Attachment 1** to the BAA posted at https://www.fbo.gov.

C. Goals and Impact: Clearly describe what the team is trying to achieve and the difference it will make (qualitatively and quantitatively) if successful. Describe the innovative aspects of the project in the context of existing capabilities and approaches, clearly delineating the uniqueness and benefits of this project in the context of the state of the art, alternative approaches, and other projects from the past and present. Describe how the proposed project is revolutionary and how it significantly rises above the current state of the art. Describe the deliverables associated with the proposed project and any plans to commercialize the technology, transition it to a customer, or further the work.

D. Technical Plan: Outline and address technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate measurable milestones (quantitative if possible) and program metrics (see Section 1.2) at intermediate stages of the program to demonstrate progress, and a plan for achieving the milestones. The technical plan should demonstrate a deep understanding of the technical challenges and present a credible (even if risky) plan to achieve the program goal. Discuss mitigation of technical risk. The technical plan should address the TA1 and TA2 proposal content requirements detailed in Section 1.1.

E. Management Plan: Provide a summary of expertise of the team, including any subcontractors, and key personnel who will be doing the work. Resumes count against the proposal page count. Identify a principal investigator for the project. Provide a clear description of the team's organization including an organization chart that includes, as applicable: the programmatic relationship of team members; the unique

capabilities of team members; the task responsibilities of team members, the teaming strategy among the team members; and key personnel with the amount of effort to be expended by each person during each year. Provide a detailed plan for coordination including explicit guidelines for interaction among collaborators/subcontractors of the proposed effort. Include risk management approaches. Describe any formal teaming agreements that are required to execute this program.

F. Capabilities: Describe organizational experience in relevant subject area(s), existing intellectual property, specialized facilities, and any Government-furnished materials or information. Discuss any work in closely related research areas and previous accomplishments.

G. Statement of Work (SOW): The SOW should provide a detailed task breakdown, citing specific tasks and their connection to the interim milestones and program metrics. Each phase of the program (Phase I base and Phase II option) should be separately defined in the SOW and each task should be identified by TA (1 or 2). The SOW must not include proprietary information.

For each task/subtask, provide:

- A detailed description of the approach to be taken to accomplish each defined task/subtask.
- Identification of the primary organization responsible for task execution (prime contractor, subcontractor(s), consultant(s), by name).
- A measurable milestone, i.e., a deliverable, demonstration, or other event/activity that marks task completion. Include quantitative metrics.
- A definition of all deliverables (e.g., data, reports, software) to be provided to the Government in support of the proposed tasks/subtasks.

H. Schedule and Milestones: Provide a detailed schedule showing tasks (task name, duration, work breakdown structure element as applicable, performing organization), milestones, and the interrelationships among tasks. The task structure must be consistent with that in the SOW. Measurable milestones should be clearly articulated and defined in time relative to the start of the project.

I. PREEMPT Transition Plan (see Section 1.2): Proposers must indicate the types of partners (e.g., government, private industry, non-profit) they plan to pursue and submit a timeline with incremental milestones toward successful engagement. Proposers should begin transition activities during the early stages of the program (Phase I). The plan should describe any potential DARPA roles. If the plan includes a start-up company, a business development strategy must be included as well.

J. PREEMPT Risk Mitigation Plan (see Section 1.4): Proposers must provide a risk mitigation plan that addresses the following:

- An assessment of potential risks to public health, agriculture, plants, animals, the environment, and national security.
- Proposed guidelines that the proposer will follow to ensure maximal biosafety and biosecurity during the course of the research.
- A communication plan that addresses content, timing, and the extent of distribution of potentially sensitive dual-use information. The plan must also address how input from DARPA, other government, and community stakeholders will be taken into account in decisions regarding communication and publication of potentially sensitive dual-use information.

K. Ethical, Legal, and Societal Implications (ELSI) (see Section 1.3): Proposers should address potential ethical, legal, and societal implications of the proposed technology.

Section III. Additional Information (Note: Does not count towards page limit)

A brief bibliography of relevant technical papers and research notes (published and unpublished) which document the technical ideas upon which the proposal is based. Copies of not more than three (3) relevant papers can be included in the submission.

a. Volume II, Cost Management Proposal

Cover Sheet (LABELED "PROPOSAL: VOLUME II"):

1. BAA number;
2. Lead Organization Submitting proposal;
3. Type of organization, selected among the following categories: "LARGE BUSINESS", "SMALL DISADVANTAGED BUSINESS", "OTHER SMALL BUSINESS", "HBCU", "MI", "OTHER EDUCATIONAL", OR "OTHER NONPROFIT";
4. Proposer's reference number (if any);
5. Other team members (if applicable), CAGE Code(s), and type of business for each;
6. Proposal title;
7. Technical point of contact (Program Manager or Principal Investigator) to include: salutation, last name, first name, street address, city, state, zip code, telephone, fax (if available), electronic mail (if available);
8. Administrative point of contact (Contracting Officer or Grant Officer) to include: salutation, last name, first name, street address, city, state, zip code, telephone, fax (if available), and electronic mail (if available);

9. Award instrument requested: cost-plus-fixed-free (CPFF), cost-contract—no fee, cost sharing contract – no fee, or other type of procurement contract (*specify*), grant, cooperative agreement, or other transaction;
10. Place(s) and period(s) of performance;
11. Total proposed cost separated by basic award and option(s) (if any);
12. Name, address, and telephone number of the proposer's cognizant Defense Contract Management Agency (DCMA) administration office (*if known)*;
13. Name, address, and telephone number of the proposer's cognizant Defense Contract Audit Agency (DCAA) audit office (*if known*);
14. Date proposal was prepared;
15. DUNS number (http://www.dnb.com/get-a-duns-number.html);
16. Taxpayer ID number (https://www.irs.gov/Individuals/International-Taxpayers/Taxpayer-Identification-Numbers-TIN);
17. CAGE code (https://www.dlis.dla.mil/bincs/FAQ.aspx);
18. Proposal validity period

Note that nonconforming proposals may be rejected without review.

Proposers that do not have a Cost Accounting Standards (CAS) complaint accounting system considered adequate for determining accurate costs that are negotiating a cost- type procurement contract must complete an SF 1408. For more information on CAS compliance, see http://www.dcaa.mil/cas.html. To facilitate this process, proposers should complete the SF 1408 found at http://www.gsa.gov/portal/forms/download/115778 and submit the completed form with the proposal. To complete the form, check the boxes on the second page, then provide a narrative explanation of your accounting system to supplement the checklist on page one. For more information, see (http://www.dcaa.mil/preaward_accounting_system_adequacy_checklist.html).

The Government strongly encourages that tables included in the cost proposal also be provided in an editable (e.g., MS Excel) format with calculation formulas intact to allow traceability of the cost proposal numbers across the prime and subcontractors.

The Government requires that the proposer provide a detailed cost breakdown to include:

(1) Total program cost broken down by Phase I (Base) and Phase II (Option) in Contractor Fiscal Year to include:
   i. Direct Labor – Including individual labor categories with associated labor hours and direct labor rates. If selected for award, be prepared to submit supporting documentation to justify labor rates. (i.e., screenshots of HR databases, comparison to NIH or other web-based salary database);
   ii. Consultants – If consultants are to be used, proposer must provide a copy of the consultant's proposed SOW as well as a signed consultant agreement or other document which verifies the proposed loaded daily / hourly rate, hours and any other proposed consultant costs (e.g., travel);

iii. Indirect Costs – Including Fringe Benefits, Overhead, General and Administrative Expense, Cost of Money, Fee, etc. (must show base amount and rate), if available, provide current Forward Pricing Rate Agreement or Forward Pricing Rate Proposal. If not available, provide 2 years historical data to include pool and expense costs used to generate the rates. For academia, provide DHHS or ONR negotiated rate package or, if calculated by other than a rate, provide University documentation identifying G&A and fringe costs by position;

iv. Travel – Provide the purpose of the trip, number of trips, number of days per trip, departure and arrival destinations, number of people, estimated rental car and airfare costs, and prevailing per diem rates as determined by gsa.gov, etc.; Quotes must be supported by screenshots from travel websites;

v. Other Direct Costs – Itemized with costs including tuition remission, animal per diem rates, health insurance/fee; back-up documentation is to be submitted to support proposed costs;

vi. Equipment Purchases – Itemization with individual and total costs, including quantities, unit prices, proposed vendors (if known), and the basis of estimate (e.g., quotes, prior purchases, catalog price lists, etc.); any item that exceeds $5,000 must be supported with back-up documentation such as a copy of catalog price lists or quotes prior to purchase (NOTE: For equipment purchases, include a letter stating why the proposer cannot provide the requested resources from its own funding), and;

vii. Materials – Itemization with costs, including quantities, unit prices, proposed vendors (if known), and the basis of estimate (e.g., quotes, prior purchases, catalog price lists, etc.); any item that exceeds $5,000 must be supported with back-up documentation such as a copy of catalog price lists or quotes prior to purchase.

(2) A summary of total program costs by major task;

(3) A summary of projected funding requirements by month;

(4) An itemization of any information technology (IT) purchase (including a letter stating why the proposer cannot provide the requested resources from its own funding), as defined in FAR Part 2.101;

(5) An itemization of Subcontracts. **All subcontractor cost proposal documentation must be prepared at the same level of detail as that required of the prime.** Subcontractor proposals should include Interdivisional Work Transfer Agreements (IWTA) or evidence of similar arrangements (an IWTA is an agreement between multiple divisions of the same organization);

(6) The source, nature, and amount of any industry cost-sharing. Where the effort consists of multiple portions which could reasonably be partitioned for purposes of funding, these should be identified as options with separate cost estimates for each;

(7) Identification of pricing assumptions of which may require incorporation into the resulting award instrument (e.g., use of Government Furnished Property/Facilities/Information, access to Government Subject Matter Expert/s, etc.);

(8) Any Forward Pricing Rate Agreement, DHHS rate agreement, other such approved rate information, or such documentation that may assist in expediting negotiations (if available); and

(9) Proposers with a Government acceptable accounting system who are proposing a cost-type contract must submit the DCAA document approving the cost accounting system.

4.2.3. Additional Proposal Information

Proprietary Markings

Proposers are responsible for clearly identifying proprietary information. Submissions containing proprietary information must have the cover page and each page containing such information clearly marked with a label such as "Proprietary" or "Company Proprietary." NOTE: "Confidential" is a classification marking used to control the dissemination of U.S. Government National Security Information as dictated in Executive Order 13526 and should not be used to identify proprietary business information.

Unclassified Submissions

DARPA anticipates that submissions received under this BAA will be unclassified. However, should a proposer wish to submit classified information, an *unclassified* email must be sent to the BAA mailbox requesting submission instructions from the Technical Office PSO. If a determination is made that the award instrument may result in access to classified information, a SCG and/or DD Form 254 will be issued by DARPA and attached as part of the award.

Human Research Subjects/Animal Use

Proposers that anticipate involving Human Research Subjects or Animal Use must comply with the approval procedures detailed at http://www.darpa.mil/work-with-us/additional-baa.

Small Business Subcontracting Plan

Pursuant to Section 8(d) of the Small Business Act (15 U.S.C. § 637(d)) and FAR 19.702(a)(1), each proposer who submits a contract proposal and includes subcontractors might be required to submit a subcontracting plan with their proposal. The plan format is outlined in FAR 19.704.

Section 508 of the Rehabilitation Act (29 U.S.C. § 749d)/FAR 39.2

All electronic and information technology acquired or created through this BAA must satisfy the accessibility requirements of Section 508 of the Rehabilitation Act (29 U.S.C. § 749d)/FAR 39.2.

Intellectual Property

All proposers must provide a good faith representation that the proposer either owns or possesses the appropriate licensing rights to all intellectual property that will be utilized under the proposed effort.

For Procurement Contracts

Proposers responding to this BAA requesting procurement contracts will need to complete the certifications at DFARS 252.227-7017. See http://www.darpa.mil/work-with-us/additional-baa for further information. If no restrictions are intended, the proposer should state "NONE."

The table below captures the requested information:

Technical Data	Summary of	Basis for	Asserted Rights	Name of Person

Computer Software To be Furnished With Restrictions	Intended Use in the Conduct of the Research	Assertion	Category	Asserting Restrictions
(LIST)	(NARRATIVE)	(LIST)	(LIST)	(LIST)

For All Non-Procurement Contracts

Proposers responding to this BAA requesting a Grant, Cooperative Agreement, Technology Investment Agreement, or Other Transaction for Prototypes shall follow the applicable rules and regulations governing these various award instruments, but, in all cases, should appropriately identify any potential restrictions on the Government's use of any Intellectual Property contemplated under the award instrument in question. This includes both Noncommercial Items and Commercial Items. Proposers are encouraged to use a format similar to that described in the section above. If no restrictions are intended, then the proposer should state "NONE."

System for Award Management (SAM) and Universal Identifier Requirements

All proposers must be registered in SAM unless exempt per FAR 4.1102. FAR 52.204-7, "System for Award Management" and FAR 52.204-13, "System for Award Management Maintenance" are incorporated into this BAA. See http://www.darpa.mil/work-with-us/additional-baa for further information.

4.2.4. Submission Information

DARPA will acknowledge receipt of all submissions and assign an identifying control number that should be used in all further correspondence regarding the submission. DARPA intends to use electronic mail correspondence regarding HR001118S0017. Submissions may not be submitted by fax or e-mail; any so sent will be disregarded.

Submissions will not be returned. An electronic copy of each submission received will be retained at DARPA and all other non-required copies destroyed. A certification of destruction may be requested, provided the formal request is received by DARPA within 5 days after notification that a proposal was not selected.

For (abstract and) proposal submission dates, see Part I., Overview Information. Submissions received after these dates and times may not be reviewed.

For Proposers Submitting Proposal Abstracts or Full Proposals as Hard Copies/On CD-ROM:

Proposers must submit an original hardcopy and one (1) electronic copy of the abstract or proposal in PDF (preferred) on a CD-ROM to the mailing address listed in Part I. Each copy must be clearly labeled with HR001118S0017, proposer organization, technical point of contact, and proposal title (short title recommended).

Please note that submitters via hardcopy/CD-ROM will still need to visit https://baa.darpa.mil to register their organization concurrently to ensure the BAA office can verify and finalize their submission.

For Proposers Submitting Proposal Abstracts or Full Proposals Requesting Procurement Contracts or OTs through DARPA's BAA Submission Portal:

Abstracts and Full Proposals sent in response to HR001118S0017 may be submitted via DARPA's BAA Website (https://baa.darpa.mil). Visit the website to complete the two-step registration process. Submitters will need to register for an Extranet account (via the form at the URL listed above) and wait for two separate e-mails containing a username and temporary password. After accessing the Extranet, submitters may then create an account for the DARPA BAA website (via the "Register your Organization" link along the left side of the homepage), view submission instructions, and upload/finalize the abstract. Proposers using the DARPA BAA Website may encounter heavy traffic on the submission deadline date; it is highly advised that submission process be started as early as possible.

All unclassified concepts submitted electronically through DARPA's BAA Website must be uploaded as zip files (.zip or .zipx extension). The final zip file should be no greater than 50 MB in size. Only one zip file will be accepted per submission. Classified submissions and proposals requesting assistance instruments (grants or cooperative agreements) should NOT be submitted through DARPA's BAA Website (https://baa.darpa.mil), though proposers will likely still need to visit https://baa.darpa.mil to register their organization (or verify an existing registration) to ensure the BAA office can verify and finalize their submission.

Technical support for BAA Website may be reached at BAAT_Support@darpa.mil, and is typically available during regular business hours, (9:00 AM- 5:00 PM EST Monday – Friday).

Proposers using the DARPA BAA Website may encounter heavy traffic on the submission deadline date; it is highly advised that submission process be started as early as possible.

For Full Proposals Requesting Cooperative Agreements:

Proposers requesting cooperative agreements may submit proposals through one of the following methods: (1) hard copy mailed directly to DARPA; or (2) electronic upload per the instructions at http://www.grants.gov/applicants/apply-for-grants.html. Cooperative agreement proposals may not be submitted through any other means. If proposers intend to use Grants.gov as their means of submission, then they must submit their entire proposal through Grants.gov; applications cannot be submitted in part to Grants.gov and in part as a hard-copy. Proposers using the Grants.gov do not submit paper proposals in addition to the Grants.gov electronic submission.

Grants.gov Submissions: Grants.gov requires proposers to complete a one-time registration process before a proposal can be electronically submitted. First time registration can take between three business days and four weeks. For more information about registering for Grants.gov, see http://www.darpa.mil/work-with-us/additional-baa.

Hard-copy Submissions: Proposers electing to submit grant or cooperative agreement proposals as hard copies must complete the SF 424 R&R form (Application for Federal Assistance,) available on the Grants.gov website http://aaply07.grants.gov/apply/forms/sample/RR_SF424_2_0-V2.0.pdf.

Failure to comply with the submission procedures may result in the submission not being evaluated. DARPA will acknowledge receipt of complete submissions via email and assign control numbers that should be used in all further correspondence regarding proposals.

4.2.5. Disclosure of Information and Compliance with Safeguarding Covered Defense Information Controls

The following provisions and clause apply to all solicitations and contracts; however, the definition of "controlled technical information" clearly exempts work considered fundamental research and therefore, even though included in the contract, will not apply if the work is fundamental research.

DFARS 252.204-7000, "Disclosure of Information"
DFARS 252.204-7008, "Compliance with Safeguarding Covered Defense Information Controls"
DFARS 252.204-7012, "Safeguarding Covered Defense Information and Cyber Incident Reporting"

The full text of the above solicitation provision and contract clauses can be found at http://www.darpa.mil/work-with-us/additional-baa#NPRPAC.

Compliance with the above requirements includes the mandate for proposers to implement the security requirements specified by National Institute of Standards and Technology (NIST) Special Publication (SP) 800-171, "Protecting Controlled Unclassified Information in Nonfederal Information Systems and Organizations" (see https://doi.org/10.6028/NIST.SP.800-171r1) that are in effect at the time the BAA is issued, or as authorized by the Contracting Officer, not later than December 31, 2017.

For awards where the work is considered fundamental research, the contractor will not have to implement the aforementioned requirements and safeguards; however, should the nature of the work change during performance of the award, work not considered fundamental research will be subject to these requirements.

4.3. FUNDING RESTRICTIONS

Not Applicable.

4.4. OTHER SUBMISSION REQUIREMENTS

Not Applicable.

5. Application Review Information

5.1. EVALUATION CRITERIA

Proposals will be evaluated using the following criteria, listed in descending order of importance: 5.1.1 Overall Scientific and Technical Merit; 5.1.2 Potential Contribution and Relevance to the DARPA Mission; and 5.1.3 Cost Realism.

5.1.1. Overall Scientific and Technical Merit

The proposed technical approach is innovative, feasible, achievable, and complete.

Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

5.1.2. Potential Contribution and Relevance to the DARPA Mission

The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security.

The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

5.1.3. Cost Realism

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.

5.2. REVIEW OF PROPOSALS

Review Process

It is the policy of DARPA to ensure impartial, equitable, comprehensive proposal evaluations based on the evaluation criteria listed in Section V.A. and to select the source (or sources) whose offer meets the Government's technical, policy, and programmatic goals.

DARPA will conduct a scientific/technical review of each conforming proposal. Conforming proposals comply with all requirements detailed in this BAA; proposals that fail to do so may be deemed non-conforming and may be removed from consideration. Proposals will not be evaluated against each other since they are not submitted in accordance with a common work statement. DARPA's intent is to review proposals as soon as possible after they arrive; however, proposals may be reviewed periodically for administrative reasons

Award(s) will be made to proposers whose proposals are determined to be the most advantageous to the Government, consistent with instructions and evaluation criteria specified in the BAA herein, and availability of funding.

Handling of Source Selection Information

DARPA policy is to treat all submissions as source selection information (see FAR 2.101 and 3.104), and to disclose their contents only for the purpose of evaluation. Restrictive notices notwithstanding, during the evaluation process, submissions may be handled by support contractors for administrative purposes and/or to assist with technical evaluation. All DARPA support contractors performing this role are expressly prohibited from performing DARPA-sponsored technical research and are bound by appropriate nondisclosure agreements. Subject to the restrictions set forth in FAR 37.203(d), input on technical aspects of the proposals may be solicited by DARPA from non-Government consultants/experts who are strictly bound by the appropriate non-disclosure requirements.

Federal Awardee Performance and Integrity Information (FAPIIS)

Per 41 U.S.C. 2313, as implemented by FAR 9.103 and 2 CFR § 200.205, prior to making an award above the simplified acquisition threshold, DARPA is required to review and consider any information available through the designated integrity and performance system (currently FAPIIS). Awardees have the opportunity to comment on any information about themselves entered in the database, and DARPA will consider any comments, along with other information in FAPIIS or other systems prior to making an award.

6. Award Administration Information

6.1. SELECTION NOTICES

As soon as the evaluation of a proposal is complete, the proposers will be notified that 1) the proposal has been selected for funding pending contract negotiations, or 2) the proposal has not been selected. These official notifications will be sent via email to the Technical POC identified on the proposal coversheet.

6.1.1. Proposal Abstracts

DARPA will respond to abstracts with a statement as to whether DARPA is interested in the idea. If DARPA does not recommend the proposer submit a full proposal, DARPA will provide feedback to the proposer regarding the rationale for this decision. Regardless of DARPA's response to an abstract, proposers may submit a full proposal. DARPA will review all full proposals submitted using the published evaluation criteria and without regard to any comments resulting from the review of an abstract.

6.1.2. Full Proposals

As soon as the evaluation of a proposal is complete, the proposer will be notified that (1) the proposal has been selected for funding pending award negotiations, in whole or in part, or (2) the proposal has not been selected. These official notifications will be sent via e-mail to the Technical POC and/or Administrative POC identified on the proposal coversheet.

6.2. ADMINISTRATIVE AND POLICY REQUIREMENTS

6.2.1. Meeting and Travel Requirements

There will be a program kickoff meeting in the Arlington, VA vicinity and all key participants are required to attend. Performers should also anticipate regular program-wide PI meetings and periodic site visits at the Program Manager's discretion to the Arlington, VA vicinity. Proposers shall include within the content of their proposal details and costs of any travel or meetings they deem to be necessary throughout the course of the effort, to include periodic status reviews by the government.

6.2.1. FAR and DFARS Clauses

Solicitation clauses in the FAR and DFARS relevant to procurement contracts and FAR and DFARS clauses that may be included in any resultant procurement contracts are incorporated herein and can be found at http://www.darpa.mil/work-with-us/additional-baa.

6.2.2. Controlled Unclassified Information (CUI) on Non-DoD Information Systems

Further information on Controlled Unclassified Information on Non-DoD Information Systems is incorporated herein can be found at http://www.darpa.mil/work-with-us/additional-baa.

6.2.3. Representations and Certifications

If a procurement contract is contemplated, prospective awardees will need to be registered in the SAM database prior to award and complete electronic annual representations and certifications consistent with FAR guidance at 4.1102 and 4.1201; the representations and certifications can be found at www.sam.gov. Supplementary representations and certifications can be found at http://www.darpa.mil/work-with-us/additional-baa.

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6.2.4. Terms and Conditions

A link to the DoD General Research Terms and Conditions for Grants and Cooperative Agreements and supplemental agency terms and conditions can be found at http://www.darpa.mil/work-with-us/contract-management#GrantsCooperativeAgreements.

6.3. REPORTING

The number and types of reports will be specified in the award document, but will include as a minimum monthly financial status reports and quarterly technical status reports. The reports shall be prepared and submitted in accordance with the procedures contained in the award document and mutually agreed on before award. Reports and briefing material will also be required as appropriate to document progress in accomplishing program metrics. A Final Report that summarizes the project and tasks will be required at the conclusion of the performance period for the award, notwithstanding the fact that the research may be continued under a follow-on vehicle.

6.4. ELECTRONIC SYSTEMS

6.4.1. Wide Area Work Flow (WAWF)

Performers will be required to submit invoices for payment directly to https://wawf.eb.mil, unless an exception applies. Performers must register in WAWF prior to any award under this BAA.

6.4.2. i-EDISON

The award document for each proposal selected for funding will contain a mandatory requirement for patent reports and notifications to be submitted electronically through i-Edison (http://public.era.nih.gov/iedison).

7. Agency Contacts

Communication via e-mail is preferred.

Points of Contact
The BAA Coordinator for this effort may be reached at:
PREEMPT@darpa.mil
DARPA/BTO
ATTN: HR001118S0017
675 North Randolph Street
Arlington, VA 22203-2114

For information concerning agency level protests see http://www.darpa.mil/work-with-us/additional-baa#NPRPAC.

8. Other Information

DARPA will host a Proposers Day in support of the PREEMPT program on **January 30, 2018**, at the Executive Conference Center in Arlington, VA. The purpose is to provide potential

proposers with information on the PREEMPT program, promote additional discussion on this topic, address questions, provide a forum to present their capabilities, and to encourage team formation.

Interested proposers are not required to attend to respond to the PREEMPT BAA, and relevant information and materials discussed at Proposers Day will be made available to all potential proposers in the form of a FAQ posted on the DARPA Opportunities Page. The event will be webcast for those who would like to participate remotely.

DARPA will not provide cost reimbursement for interested proposers in attendance.

An online registration form and various other meeting details can be found at the registration website, https://events.sa-meetings.com/PREEMPTProposersDay.

To encourage team formation, interested proposers are encouraged to submit information to be shared with all potential proposers through the Proposers Day website and the DARPA Opportunities Page. This information may include contact information, relevant publications, and a slide or poster to summarize the proposer's interests.

Participants are required to register no later than **January 23, 2018**, for physical attendance, and **January 26, 2018**, for the webcast. This event is not open to the Press. The Proposers Day will be open to members of the public who have registered in advance for the event; **there will be no onsite registration**.

All foreign nationals, including permanent residents, must complete and submit a DARPA Form 60 "Foreign National Visit Request," which will be provided in the registration confirmation email.

Proposers Day Point of Contact: DARPA-SN-18-18@darpa.mil.

9. Appendix 1 – Volume II checklist

Volume II, Cost Proposal
Checklist and Sample Templates

The following checklist and sample templates are provided to assist the proposer in developing a complete and responsive cost volume. Full instructions appear in Section 4.2.2 beginning on Page 25 of HR001118S0017. This worksheet must be included with the coversheet of the Cost Proposal.

1. Are all items from Section 4.2.2 (Volume II, Cost Proposal) of **HR001118S0017** included on your Cost Proposal cover sheet?
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]
   If reply is "No", please explain:

2. Does your Cost Proposal include (1) a summary cost buildup by Phase, (2) a summary cost buildup by Year, and (3) a detailed cost buildup of for each Phase that breaks out each task and shows the cost per month?
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

   If reply is "No", please explain:

3. Does your cost proposal (detailed cost buildup #3 above in item 2) show a breakdown of the major cost items listed below:
   Direct Labor (Labor Categories, Hours, Rates)
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

   Indirect Costs/Rates (i.e., overhead charges, fringe benefits, G&A)
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

   Materials and/or Equipment
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

   Subcontracts/Consultants
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

   Other Direct Costs
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

   Travel
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

   If reply is "No", please explain:

4. Have you provided documentation for proposed costs related to travel, to include purpose of trips, departure and arrival destinations and sample airfare?
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

If reply is "No", please explain:

5. Does your cost proposal include a complete itemized list of all material and equipment items to be purchased (a priced bill-of-materials (BOM))?
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

   If reply is "No", please explain:

6. Does your cost proposal include vendor quotes or written engineering estimates (basis of estimate) for all material and equipment with a unit price exceeding $5000?
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

   If reply is "No", please explain:

7. Does your cost proposal include a clear justification for the cost of labor (written labor basis-of-estimate (BOE)) providing rationale for the labor categories and hours proposed for each task?
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

   If reply is "No", please explain:

8. Do you have subcontractors/consultants? If YES, continue to question 9. If NO, skip to question 13.
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

9. Does your cost proposal include copies of all subcontractor/consultant technical (to include Statement of Work) and cost proposals?
   ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

   If reply is "No", please explain:

10. Do all subcontract proposals include the required summary buildup, detailed cost buildup, and supporting documentation (SOW, Bill-of-Materials, Basis-of-Estimate, Vendor Quotes, etc.)?
    ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

    If reply is "No", please explain:

11. Does your cost proposal include copies of consultant agreements, if available?
    ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

    If reply is "No", please explain:

12. If requesting a FAR-based contract, does your cost proposal include a tech/cost analysis for all proposed subcontractors?
    ○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

    If reply is "No", please explain:

13. Have all team members (prime and subcontractors) who are considered a Federally Funded Research & Development Center (FFRDC), included documentation that clearly demonstrates work is not otherwise available from the private sector AND provided a letter on letterhead from the sponsoring organization citing the specific authority establishing their eligibility to propose to government solicitations and compete with industry, and compliance with the associated FFRDC sponsor agreement and terms and conditions.

○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

If reply is "No", please explain:

14. Does your proposal include a response regarding Organizational Conflicts of Interest?

○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

If reply is "No", please explain:

15. Does your proposal include a completed Data Rights Assertions table/certification?

○ **YES** ○ **NO** **Appears on Page(s)** [Type text]

If reply is "No", please explain:

Re: Support letter (PREEMPT)

Sleeman, Jonathan M <jsleeman@usgs.gov>

Tue 3/20/2018 4:25 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Richgels, Katherine L <krichgels@usgs.gov>

Hi Tonie,

The authority we should cite is 31 U.S.C 1535A Economy Act. If you wanted to finalize the letter I will sign it.

FYI, Lisa Meicher researched this for us.

Best wishes,

Jonathan

On Tue, Mar 20, 2018 at 2:11 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> This is all the response I got regarding my eligibility question. -Tonie
> ---------- Forwarded message ----------
> From: **Luke Hamel** <hamel@ecohealthalliance.org>
> Date: Tue, Mar 20, 2018 at 2:05 PM
> Subject: Re: Support letter (PREEMPT)
> To: "Rocke, Tonie" <trocke@usgs.gov>
>
> Hi Tonie,
>
> I apologize that the link did not provide helpful guidance. I've spoken with fellow staff at EHA and they recommend speaking with either: (1) The director or other representative of NWHC's grant department (if your institution has such a department), or (2) Someone in NWHC's Operations or Finance department.
>
> My colleague does not think DARPA will give additional guidance regarding this matter. Please let me know if this information helps.
>
> Best,
>
> **Luke Hamel**
> *Program Assistant*
>
> EcoHealth Alliance
> 460 West 34th Street – 17th floor
> New York, NY 10001

(b) (6) (direct)
(mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Tue, Mar 20, 2018 at 1:52 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Luke: I have no idea how to respond about the eligibility. Is there someone we can call at DARPA. The link you provided was no help at all and in fact includes no DOI agencies. -Tonie
>
> On Tue, Mar 20, 2018 at 12:26 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > Please see the required **collaborator support letter (attached) and comments within.** Thank you for your patience and please let me know if you have any questions.
> >
> > Best,
> >
> > **Luke Hamel**
> > *Program Assistant*
> >
> > EcoHealth Alliance
> > 460 West 34th Street – 17th floor
> > New York, NY 10001
> >
> > (direct)
> > (b) (6) (mobile)
> > www.ecohealthalliance.org
> >
> > ***EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.***

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

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Tonie E. Rocke
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trocke@usgs.gov

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Jonathan Sleeman, MA, VetMB, Dipl. ACZM, Dipl. ECZM, MRCVS
Center Director
USGS, National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711

Tel: (608) 270 2401
Fax: (608) 270 2415
Email: jsleeman@usgs.gov

The USGS National Wildlife Health Center's mission is to safeguard wildlife and ecosystem health through dynamic partnerships and exceptional science

OIE Collaborating Centre for Research, Diagnosis and Surveillance of Wildlife Pathogens

Re: DARPA PRE-EMPT

Anna Willoughby <willoughby@ecohealthalliance.org>

Tue 3/20/2018 7:51 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Hi Tonie,

Thanks for these bios, they are great. I would wait to add detailed edits until the updated draft. Peter has been dedicating this week to writing and editing, so things may have been reformatted in the new draft. If you have important items that need editing/adding that you'd like to address before your travel, feel free to send as a separate note or we can discuss on the call tomorrow. I apologize if this is unclear, Peter will have good advice on this tomorrow.

Best,
Anna

On Tue, Mar 20, 2018 at 5:20 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Anna: Should I wait for Peter's updated draft before I make revisions? Also, attached is a file containing bios for both Rachel Abbott and myself. Please let me know if you think they are sufficient. Best -Tonie
>
> On Tue, Mar 20, 2018 at 4:15 PM, Anna Willoughby <willoughby@ecohealthalliance.org> wrote:
>
> > Dear all,
> >
> > Please find the NWHC section of the proposal. Peter is currently working on an updated draft, but this should be sufficient for composing your budget and particular task. Please let me know if you have any questions. If needed, I am also attaching the original BAA for the program, with PARC's focus being TA2.
> >
> > When can we expect the revised budget and scope of work?
> >
> > Best,
> > Anna
> >
> > On Fri, Mar 16, 2018 at 3:12 PM, Anna Willoughby <willoughby@ecohealthalliance.org> wrote:
> >
> > > Thanks for these details, Jerome. Attached are my notes from the call. Action items include:
> > >
> > > *- Jerome to send more detailed scope of work with paragraphs and revised budget by early next week*
> > > *- EHA will send PARC the NWHC section of the proposal on Monday*
> > > *- EHA will send the format of letter of support for PARC*
> > > *- EHA to follow up with Kateri with requested information*
> > >
> > > For your question on collaborating with other institutes, it is likely that all organizations involved may have insight into the aerosol-bat interaction. I believe this topic would be covered during the

Annual Meeting between all partners, as well as during relevant cross-partner trips, in addition to monthly conference calls.

Please let us know if you have further questions.

Best,
Anna

On Fri, Mar 16, 2018 at 2:57 PM, <Jerome.Unidad@parc.com> wrote:

> An additional point for Peter, Tonie (and everyone),
>
> For the spray technology, refinement of the details with respect to aerosol-bat interaction (i.e. the preliminary field testing to see how bats react to the aerosol) and eventual field-deployment in China, will the technical lead for coordinating this segment of the project be USGS – National Wildlife Center? Or should we also expect to work/coordinate with other institutes who would give feedback and insights on how this works?
>
> Thanks. This is just for our information.
>
> Best,
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company
>
> ---
>
> **From:** Unidad, Jerome <Jerome.Unidad@parc.com>
> **Sent:** Friday, March 16, 2018 11:52 AM
> **To:** 'William B. Karesh' <karesh@ecohealthalliance.org>; 'Peter Daszak' <daszak@ecohealthalliance.org>
> **Cc:** 'Luke Hamel' <hamel@ecohealthalliance.org>; 'Anna Willoughby' <willoughby@ecohealthalliance.org>; 'Alison Andre' <andre@ecohealthalliance.org>; 'Amanda Andre' <amanda.andre@ecohealthalliance.org>; 'Rocke, Tonie' <trocke@usgs.gov>; Paul, Kateri

<Kateri.Paul@parc.com> <Kateri.Paul@parc.com>
Subject: RE: DARPA PRE-EMPT

Peter and team,

I'm currently working on putting together a revised budget and equivalent statement of work (tasks breakdown) for PARC's involvement with the project. You can expect this about early next week – approximately Monday. Officially, for the submission, our capture manager, Kateri Paul, who takes care of the other things would need the following things from your equivalent to facilitate our parts of the submission.

1. Request for Proposal that we can respond to with what they need for their package to DARPA
2. Start date of the proposed effort
3. Contract or a Grant/Other Transaction

Once we have finalized the scope of work and the budget, Kateri will be in touch for these other aspects. Her contact information can be found below.

Kateri E. Paul

Capture Manager, Public Sector

Global Business Development

Palo Alto Research Center (PARC)

3333 Coyote Hill Road

Palo Alto, CA 94304

Kateri.Paul@parc.com

650-812-4821 (desk)

617-596-2023 (mobile)

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Unidad, Jerome <Jerome.Unidad@parc.com>
Sent: Thursday, March 15, 2018 3:33 PM
To: 'Rocke, Tonie' <trocke@usgs.gov>; William B. Karesh <karesh@ecohealthalliance.org>; Johnson, David <David.Johnson@parc.com> <David.Johnson@parc.com>
Cc: Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: RE: DARPA PRE-EMPT

Dear all,

10AM-11AM PST (12PM-1PM CT, 1PM-2PM ET) should work for us. I shall setup a WebEx meeting for this, given the number of participants.

Let me know if this timeslot will work.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 2:39 PM
To: William B. Karesh <karesh@ecohealthalliance.org>
Cc: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I assume that is ET? -T

On Thu, Mar 15, 2018 at 4:14 PM, William B. Karesh <karesh@ecohealthalliance.org> wrote:

> Tonie and Jerome,
>
> We would still like to speak. Anytime on Friday between 11:00 AM and 2:00 PM would be great.
>
> BK

William B. Karesh, D.V.M

Executive Vice President for Health and Policy

EcoHealth Alliance

460 West 34th Street - 17th Floor

New York, NY 10001 USA

+1.212.380.4463 (direct)

+1.212.380.4465 (fax)

www.ecohealthalliance.org

President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

On Mar 15, 2018, at 4:55 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

Hi all: Since we didn't hear back from EcoHealth Alliance, Jerome and I went ahead with a short call we had been planning anyway regarding some technical details. I told him our concerns about the proposed budget and we think we have a pretty good plan to reduce the scope of work to the funds we have available. PARC is very unique in developing this technology and their technology fits very well with other work I am doing, so we both feel pretty confident we can work something out. If you still wish to have a discussion among all of us, we can schedule that for tomorrow, as I believe Jerome had another meeting to run off to for the rest of the day. I'm available the rest of the day if you wish to chat about this in person. Best - Tonie

On Thu, Mar 15, 2018 at 3:42 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

> Actually – can we do a phone call – I'll be driving. 5.15pm would be perfect (NYC time), Today Thursday.
>
> Is that possible?
>
> Our call in line is: 1-719-785-9461
>
> Passcode: 9784#
>
> Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4474

www.ecohealthalliance.org

@PeterDaszak

@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Jerome.Unidad@parc.com [mailto:Jerome.Unidad@parc.com]
Sent: Thursday, March 15, 2018 4:23 PM
To: trocke@usgs.gov
Cc: William B. Karesh; Peter Daszak; Luke Hamel
Subject: RE: DARPA PRE-EMPT

I can setup a WebEx quickly if we will have multiple parties.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 1:22 PM
To: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Daszak Peter <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I'm available as well. Billy, do you have a call in number? -Tonie

On Thu, Mar 15, 2018 at 3:20 PM, <Jerome.Unidad@parc.com> wrote:

> Dear all,
>
> Sorry for the late response – yes, I will be available for a phone call now. Up to 2PM.
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company
>
> ---
>
> **From:** William B. Karesh [mailto:karesh@ecohealthalliance.org]
> **Sent:** Thursday, March 15, 2018 12:49 PM
> **To:** Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
> **Cc:** Rocke, Tonie <trocke@usgs.gov>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
> **Subject:** DARPA PRE-EMPT

Dear Dr. Unidad,

Thanks for your quick responses to Dr. Rocke. Would you be available for a short call with Dr. Daszak, Dr. Rocke and me this afternoon or Friday.

We're on tight timeline so we thought a phone call might be save quite a bit of time.

Thanks in advance,

Billy

William B. Karesh, D.V.M

Executive Vice President for Health and Policy

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--

Tonie E. Rocke

USGS National Wildlife Health Center

6006 Schroeder Rd.

Madison, WI 53711

608-270-2451

trocke@usgs.gov

--

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USGS National Wildlife Health Center

6006 Schroeder Rd.

Madison, WI 53711

608-270-2451

trocke@usgs.gov

--

Tonie E. Rocke

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6006 Schroeder Rd.

Madison, WI 53711

608-270-2451

trocke@usgs.gov

--

Anna Willoughby

Research Assistant

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(b) (6) (cell)

www.ecohealthalliance.org

Visit our blog: http://blog.ecohealthalliance.org/updates

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--

Anna Willoughby

Research Assistant

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--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

--

Anna Willoughby
Research Assistant

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With this science, we develop solutions that prevent pandemics and promote conservation.

PREEMPT budget - Local ground travel

Luke Hamel <hamel@ecohealthalliance.org>

Wed 3/21/2018 10:43 AM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Jonathon Musser <musser@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>

Hi Tonie,

Previously, you asked me about how to estimate costs for local ground transportation in China. For this, we typically estimate the cost of a local taxi fare from the airport to either: (1) a hotel or (2) the meeting venue (which in this case would be the Wuhan Institute of Virology).

We have found that the following website, is very helpful for estimating local taxi fares. For the departure point, you can use 'Wuhan Tianhe International Airport' and for the destination, 'Wuhan Institute of Virology.' You may also need to enter 'Beijing, China' into the top field that reads, *Find your taxi calculator*.

Lastly, we typically assume that one taxi will accommodate up to 3 passengers. So if there are 1 or 2 others traveling with you from NWHC to China, there is no need to account for additional taxi fares.

Please forward this information along to the travel coordinator who has been assisting you, and let me know if you experience any difficulties with the site.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(b) (6) (mobile)
www.ecohealthalliance.org

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Re: Identification of pricing assumptions (PREEMPT)

Luke Hamel <hamel@ecohealthalliance.org>

Wed 3/21/2018 10:45 AM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Meicher, Lisa K <lmeicher@usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>

Hi Tonie,

Great to hear about the status of the support letter! I will look into additional information for the 'pricing assumptions' and get back to you as soon as possible.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(b) (6) (mobile)
www.ecohealthalliance.org

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On Wed, Mar 21, 2018 at 10:16 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

> We're not exactly sure what "identification of pricing assumptions" means. Do you have any other information? On the plus side, we did figure out the eligibility question and the letter you requested will be on its way to you shortly. -Tonie
> ---------- Forwarded message ----------
> From: **Meicher, Lisa** <lmeicher@usgs.gov>
> Date: Wed, Mar 21, 2018 at 8:53 AM
> Subject: Re: Identification of pricing assumptions (PREEMPT)
> To: "Rocke, Tonie" <trocke@usgs.gov>
> Cc: "Richgels, Katherine" <krichgels@usgs.gov>
>
> Tonie,
>
> To be honest, I'm not exactly sure. If they mean costs related to facilities, that is normally covered by the burden fee. I would need future clarification.

Lisa K. Meicher
Budget Analyst
USGS National Wildlife Health Center
6006 Schroeder Rd
Madison, WI 53711
608-270-2410
fax 608-270-2415
lmeicher@usgs.gov

On Tue, Mar 20, 2018 at 4:31 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Lisa: Do you know what they are talking about below and do we need to address this? Thanks -Tonie
> ---------- Forwarded message ----------
> From: **Luke Hamel** <hamel@ecohealthalliance.org>
> Date: Tue, Mar 20, 2018 at 3:59 PM
> Subject: Identification of pricing assumptions (PREEMPT)
> To: "Rocke, Tonie" <trocke@usgs.gov>
>
> Hi Tonie,
>
> Please see below, an additional item that we will need to address. Please forward this text to whomever you have been speaking with at NWHC regarding financial guidance for the subaward. They should have a good idea of what this entails for your institution, and this is information that, if relevant, we will need to include within your budget justification.
>
> Taken from p. 27 of the PREEMPT BAA:
>
> "(7) Identification of pricing assumptions of which may require incorporation into the resulting award instrument (e.g., use of Government Furnished Property/Facilities/Information, access to Government Subject Matter Expert/s, etc.)"
>
> Please let me know if you have any questions regarding this matter.
>
> Best,
>
> **Luke Hamel**
> *Program Assistant*
>
> EcoHealth Alliance
> 460 West 34th Street – 17th floor
> New York, NY 10001
>
> (b) (6) (direct)
> (mobile)
> www.ecohealthalliance.org
>
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> ***ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.***
>
> --
> Tonie E. Rocke
> USGS National Wildlife Health Center
> 6006 Schroeder Rd.
> Madison, WI 53711
> 608-270-2451
> trocke@usgs.gov

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

Re: PREEMPT - Registering on Grants.gov

Luke Hamel <hamel@ecohealthalliance.org>

Wed 3/21/2018 1:03 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Great. Thank you, Tonie.

Luke Hamel
Program Assistant

EcoHealth Alliance
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New York, NY 10001

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www.ecohealthalliance.org

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On Wed, Mar 21, 2018 at 1:06 PM, Rocke, Tonie <trocke [redacted] ov> wrote:

> I already have an account. Username: (b) (6)
>
> On Wed, Mar 21, 2018 at 11:43 AM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > In order to submit our PREEMPT proposal, any individual listed as 'Key Personnel' on our project must create an account on Grants.gov (the website that we will use for submission).
> >
> > As you are considered 'Key Personnel' for our proposal, **I ask that you create an account as soon as possible, using this link.**
> >
> > This should be quick and easy to do, as applicants are only required to provide the following information:
> > NAME
> > EMAIL
> > PHONE NUMBER
> > USERNAME
> > PASSWORD
> > SECURITY QUESTION
> >
> > Please note that if you have previously created an account on Grants.gov, there is no need to register again. **In either case however, I will need you to share your username with me** so that I can add you to our team profile on the Grants.gov website.
> >
> > Please let me know if you have any questions.
> >
> > Best,

Re: [Reminder] Call today @ 6 PM (ET)

Anna Willoughby <willoughby@ecohealthalliance.org>

Wed 3/21/2018 4:25 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Luke Hamel <hamel@ecohealthalliance.org>; Dr. Peter Daszak <daszak@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>

Hi Tonie,

In advance of our call, please find updated budget files (pdf form and excel breakdown). Overall numbers haven't changed much, an increase of ~$10,000. New changes are 1) Updated your time slightly to match travel, 2) Updated travel break down to include per diems per person and formulas, and 3) added materials by year formulas. I am also attaching a performance schedule that shows when (approximately) you would be traveling and hosting partners (to justify your time). I will be on the call to clarify all these items and answer any questions you may have.

Best,
Anna

On Wed, Mar 21, 2018 at 5:14 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Hi Tonie,
>
> This is just a reminder that we have a **PREEMPT call scheduled today at 6 PM (ET)/5 PM (CT)**. Please use the following number and password to join the call:
>
> Phone: 1-719-785-9461
> Password: 9784#
>
> Best,
>
> **Luke Hamel**
> *Program Assistant*
>
> EcoHealth Alliance
> 460 West 34th Street – 17th floor
> New York, NY 10001
>
> (b) (6) (direct)
> (b) (6) (mobile)
> www.ecohealthalliance.org
>
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--

Anna Willoughby

Research Assistant

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Lead	Purpose	Personnel	Phase I																								Phase II																	
			Month																																									
			1	2	3	4	5	6	7	8	9	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#
	DARPA meeting	Dr. Rocke	KO																																									
NWHC	Annual meeting	Dr. Rocke; Dr. Abbott (Y1,3,OY2)											NY												CN												NY						NY	
	US site visit	Dr. Rocke; 2 Students							■												■												■											
	China site visit	Dr. Rocke										■																							■									
EHA	Partner Visit to NWHC	Dr. Epstein; Dr. Ross				■												■											■															
PARC	Partner Visit to NWHC	PARC						■												■											■													

WORKSPACE FORM

1-800-518-4726
SUPPORT@GRANTS.GOV

This Workspace form is one of the forms you need to complete prior to submitting your Application Package. This form can be completed in its entirety offline using Adobe Reader. You can save your form by clicking the "Save" button and see any errors by clicking the “Check For Errors” button. In-progress and completed forms can be uploaded at any time to Grants.gov using the Workspace feature.

When you open a form, required fields are highlighted in yellow with a red border. Optional fields and completed fields are displayed in white. If you enter invalid or incomplete information in a field, you will receive an error message. Additional instructions and FAQs about the Application Package can be found in the Grants.gov Applicants tab.

OPPORTUNITY & PACKAGE DETAILS:

Opportunity Number:	HR001118S0017
Opportunity Title:	PREventing EMerging Pathogenic Threats
Opportunity Package ID:	PKG00237724
CFDA Number:	12.910
CFDA Description:	Research and Technology Development
Competition ID:	
Competition Title:	
Opening Date:	01/19/2018
Closing Date:	03/27/2018
Agency:	DARPA - Biological Technologies Office
Contact Information:	BAA Coordinator PREEMPT@darpa.mil

APPLICANT & WORKSPACE DETAILS:

Workspace ID:	WS00094394
Application Filing Name:	Project DEFUSE
DUNS:	0770900660000
Organization:	ECOHEALTH ALLIANCE INC.
Form Name:	R & R Subaward Budget 10 YR Subform
Form Version:	1.4
Subform Name:	USGS Ntl. Wildlife Health Cen
Requirement:	Optional
Download Date/Time:	Mar 06, 2018 05:28:38 PM EST
Form State:	Error(s)

FORM ACTIONS:

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 1** **Start Date:** 12/01/2018 **End Date:** 11/30/2019

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.85			9,179.00	2,475.00	11,654.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 88,630.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00	0.00	24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel 24,782.00

Total Salary, Wages and Fringe Benefits (A+B) 113,412.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	7,689.00
2. Foreign Travel Costs	3,384.00
Total Travel Cost	11,073.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	21,982.52
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.	Rabies prophylaxis	4,020.00
10.		
	Total Other Direct Costs	38,602.52

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	163,087.52

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	163,087.52	105,256.69

Total Indirect Costs 105,256.69

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	268,344.21

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	268,344.21

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 2** **Start Date:** 12/01/2019 **End Date:** 11/30/2020

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.60			6,479.00	1,747.00	8,226.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 85,202.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00	0.00	24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel 24,782.00

Total Salary, Wages and Fringe Benefits (A+B) 109,984.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	2,316.00
2. Foreign Travel Costs	8,245.50
Total Travel Cost	10,561.50

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	17,976.52
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.	Rabies prophylaxis	4,020.00
10.		
	Total Other Direct Costs	34,596.52

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	155,142.02

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	155,142.00	100,128.65

Total Indirect Costs 100,128.65

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	255,270.67

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	255,270.67

L. Budget Justification

(Only attach one file.) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 3** **Start Date:** 12/01/2020 **End Date:** 11/30/2021

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.80			8,639.00	2,329.00	10,968.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 87,944.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00	0.00	24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel: 24,782.00

Total Salary, Wages and Fringe Benefits (A+B): 112,726.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	6,118.00
2. Foreign Travel Costs	3,384.00
Total Travel Cost	9,502.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	17,976.52
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.	Rabies prophylaxis	4,020.00
10.		
	Total Other Direct Costs	34,596.52

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	156,824.52

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	156,824.52	101,214.21

Total Indirect Costs 101,214.21

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	258,038.73

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	258,038.73

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 4

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 4** **Start Date:** 12/01/2021 **End Date:** 03/31/2022

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.40			4,319.00	1,165.00	5,484.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	6.00			30,502.00	7,986.00	38,488.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 43,972.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						

Total Number Other Personnel

Total Other Personnel

Total Salary, Wages and Fringe Benefits (A+B) 43,972.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	4,167.00
2. Foreign Travel Costs	
Total Travel Cost	4,167.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	2,163.43
2.	Publication Costs	6,000.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.		
9.		
10.		
	Total Other Direct Costs	8,163.43

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	56,302.43

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	56,302.43	36,337.50

Total Indirect Costs 36,337.50

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	92,639.93

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	92,639.93

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		305,748.00
Section B, Other Personnel		74,346.00
Total Number Other Personnel	9	
Total Salary, Wages and Fringe Benefits (A+B)		380,094.00
Section C, Equipment		
Section D, Travel		35,303.50
1. Domestic	20,290.00	
2. Foreign	15,013.50	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		115,958.99
1. Materials and Supplies	60,098.99	
2. Publication Costs	6,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	37,800.00	
9. Other 2	12,060.00	
10. Other 3		
Section G, Direct Costs (A thru F)		531,356.49
Section H, Indirect Costs		342,937.05
Section I, Total Direct and Indirect Costs (G + H)		874,293.54
Section J, Fee		
Section K, Total Costs and Fee (I + J)		874,293.54

TRAVEL

Trip #:	1	Location:	Arlington, VA, USA	Contract Period
Purpose:	DARPA Kickoff Meeting			Base 1

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
1.75	1	$333.00	$69.00	$250.00	$120.00	$1,011.25

Itemized Expenses for "Other"

Description	Amount
Parking	$20.00
Transportation to/from airport and in Arlington	$100.00
Total:	$120.00

Trip #:	2	Location:	Kunming, Yunnan, China	Contract Period
Purpose:	China Cave Site Visit			Base 1

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
7	1	$1,370.00	$115.00	$147.00	$180.00	$3,384.00

Itemized Expenses for "Other"

Description	Amount
Parking	$80.00
Transportation to/from airport and in Arlington	$100.00
Total:	$180.00

Trip #:	3	Location:	Upper Peninsula Michagan	Contract Period
Purpose:	US Cave Site Visit			Base 1

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
4	3	$0.00	$51.00	$93.00	$588.00	$2,316.00

Itemized Expenses for "Other"

Description	Amount
Gas	$120.00
Government Car Use	$468.00
Total:	$588.00

Trip #:	4	Location:	New York, NY, USA	Contract Period
Purpose:	Annual Meeting (Rocke + Abbott)			Base 1

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
3	2	$666.00	$74.00	$291.00	$140.00	$4,362.00

Itemized Expenses for "Other"

Description	Amount
Parking	$40.00
Transportation to/from airport and in New York	$100.00
Total:	$140.00

Trip #:	5	Location:	Upper Peninsula, Michigan, USA	Contract Period
Purpose:	US Cave Site Visit			Base 2

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
4	3	$0.00	$51.00	$93.00	$588.00	$2,316.00

Itemized Expenses for "Other"

Description	Amount
Gas	$120.00
Government Car Use	$468.00
Total:	$588.00

Trip #:	6	Location:	Wuhan, China	Contract Period
Purpose:	Annual Meeting (Rocke)			Base 2

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
4.75	1	$6,861.00	$115.00	$147.00	$140.00	$8,245.50

Itemized Expenses for "Other"

Description	Amount
Parking	$40.00
Transportation to/from airport in Wuhan	$100.00
Total:	$140.00

Trip #:	7	Location:	Upper Peninsula, Michigan, USA	Contract Period
Purpose:	US Cave Site Visit			Option I

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
4	3	$0.00	$51.00	$93.00	$588.00	$2,316.00

Itemized Expenses for "Other"

Description	Amount
Gas	$120.00
Government Car Use	$468.00
Total:	$588.00

Trip #:	8	**Location:**	Kunming, Yunnan, China	**Contract Period**	
Purpose:	Deployment Visit			Option I	

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
7	1	$1,370.00	$115.00	$147.00	$180.00	$3,384.00

Itemized Expenses for "Other"

Description	Amount
Parking	$80.00
Transportation to/from airport and in Kunming	$100.00
Total:	$180.00

Trip #:	9	**Location:**	New York, NY, USA	**Contract Period**	
Purpose:	Annual Meeting (Rocke + Abbott)			Option I	

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
3	2	$666.00	$74.00	$291.00	$140.00	$3,802.00

Itemized Expenses for "Other"

Description	Amount
Parking	$40.00
Transportation to/from airport	$100.00
Total:	$140.00

Trip #:	10	**Location:**	New York, NY, USA	**Contract Period**	
Purpose:	Annual Meeting (Rocke + Abbott)			Option II	

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
4	1	$666.00	$74.00	$291.00	$140.00	$2,266.00
3	1	$666.00	$74.00	$291.00	$140.00	$1,901.00

Itemized Expenses for "Other"

Description	Amount
Parking	$40.00
Transportation to/from airport and in New York	$100.00

	Total:	$140.00

MATERIALS/EQUIPMENT

Item	Manufacturer	Part Number	Unit Price	Quantity	Total Price	Contract Period	Additional Information
Harp Trap	Bat conservation and management		$2,003	2	$4,006.00	Y1	
Mealworms	Rainbow mealworms		$100/20,000	12	$1,200.00	Y1-Y3	
bat caging materials	various		$500/cage	9	$4,500.00	Y1-Y3	custom made
bat wing bands	Porzana		$596/box	9	$4,768.00	Y1-Y3	
Cut resistant gloves	Varied		$15/pr	30	$450.00	Y1-Y3	
Tyvek suits	DuPOnt	EV29135313	$306/case	15	$4,590.00	Y1-Y3	
Tyvek aprons	Lakeland	6EHH7	$58/case	15	$870.00	Y1-Y3	
N95 respirators	3M	9511	$20/box	45	$900.00	Y1-Y3	
PAPRs replacement covers	3M		$96/3 units	45	$4,320.00	Y1-Y3	
cell culture flasks	Corning	430641U	415/case	5	$2,075.00	Y1-Y3	
cell culture flasks	Corning	431080	425/case	10	$4,250.00	Y1-Y3	
Nunc cell factories	Nunc	140250	$370/case	12	$4,440.00	Y1-Y3	
fetal bovine serum	GE Hyclone	SH30071.03	$600/bottle	8	$4,800.00	Y1-Y3	
DMEM medium	GE Hyclone	SH30021.02	$30/l	10	$300.00	Y1-Y3	
Selamectin	Zoetis		$250		$250.00	Y1-Y3	
glycerin jelly	Carolina Biological Supply		$43 bottle	50	$2,150.00	Y1-Y3	
rhodamine B	Sigma		$56/100g	6	$336.00	Y1-Y3	
hair collection bags	U-line		$75/box	10	$750.00	Y1-Y3	
96 well plates	Corning	3599	$600/case	8	$4,800.00	Y1-Y3.5	
pipette tips	Fisher	13-676-10	$100/case	50	$5,000.00	Y1-Y3.5	
Consumables	miscellaneous				$5,344.00	Y1-Y3.5	needles, syringes,whirl paks, plastic bags, other disposables, all <5K
				Total	$60,099.00		

Y1 Total	$21,982.52
Y2 Total	$17,976.52
Y3 Total	$17,976.52
Y3.5 Total	$2,163.43

OTHER DIRECT COSTS

Description	Total Price	Contract Period	Additional Information
animal perdiem costs	$12,600	Base 1	up to 60 bats for 120 days at $105/day in BSL3 animal facility, includes daily husbandry, gut-loading meal worms, cleaning cages, feeding bats, veterinary services and daily surcharge for rom use,
animal perdiem costs	$12,600	Base 2	up to 60 bats for 120 days at $105/day in BSL3 animal facility (ame as above)
animal perdiem costs	$12,600	Option 1	up to 60 bats for 120 days at $105/day in BSL3 animal facility (same as above)
rabies prphylactic shots	$4,020	Base 1	all animal care and technical staff must be vaccinated against rabies to work with bats. 1005/person
rabies prphylactic shots	$4,020	Base 2	all animal care and technical staff must be vaccinated against rabies to work with bats. 1005/person
rabies prphylactic shots	$4,020	Option 1	all animal care and technical staff must be vaccinated against rabies to work with bats. 1005/person
Total	$49,860		

Re: [Reminder] Call today @ 6 PM (ET)

Anna Willoughby <willoughby@ecohealthalliance.org>

Wed 3/21/2018 4:47 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Yes, I updated this in the budget as well. We are planning 12/1/18.

On Wed, Mar 21, 2018 at 5:44 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Anna: Just out of curiosity, what is the projected start date for the project? I was using 10/1/18. -T
>
> On Wed, Mar 21, 2018 at 4:25 PM, Anna Willoughby <willoughby@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > In advance of our call, please find updated budget files (pdf form and excel breakdown). Overall numbers haven't changed much, an increase of ~$10,000. New changes are 1) Updated your time slightly to match travel, 2) Updated travel break down to include per diems per person and formulas, and 3) added materials by year formulas. I am also attaching a performance schedule that shows when (approximately) you would be traveling and hosting partners (to justify your time). I will be on the call to clarify all these items and answer any questions you may have.
> >
> > Best,
> > Anna
> >
> > On Wed, Mar 21, 2018 at 5:14 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
> >
> > > Hi Tonie,
> > >
> > > This is just a reminder that we have a **PREEMPT call scheduled today at 6 PM (ET)/5 PM (CT)**.
> > > Please use the following number and password to join the call:
> > >
> > > Phone: 1-719-785-9461
> > > Password: 9784#
> > >
> > > Best,
> > >
> > > **Luke Hamel**
> > > *Program Assistant*
> > >
> > > EcoHealth Alliance
> > > 460 West 34th Street – 17th floor
> > > New York, NY 10001
> > >
> > > (b) (6) (direct)

(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

--

Anna Willoughby
Research Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
1.212.380.4465 (fax)
(b) (6) (cell)

www.ecohealthalliance.org

Visit our blog: http://blog.ecohealthalliance.org/updates

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

--

Anna Willoughby

Research Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
1.212.380.4465 (fax)
(b) (6) (cell)

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Visit our blog: http://blog.ecohealthalliance.org/updates

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

Re: letter for Jonathan to sign

Luke Hamel <hamel@ecohealthalliance.org>

Wed 3/21/2018 10:15 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>

Thank you, Tonie. I hope you enjoy your trip!

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Wed, Mar 21, 2018 at 5:54 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> OK thanks, since I will be out of the office until Monday, if you need something before then, either contact Rachel or Katie (both copied here). -Tonie
>
> On Wed, Mar 21, 2018 at 4:44 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Wonderful. Thank you, Tonie. Regarding the 'pricing agreement', we have reached out to DARPA staff for further clarification and will contact you once we've received a response.
> >
> > Best,
> >
> > **Luke Hamel**
> > *Program Assistant*
> >
> > EcoHealth Alliance
> > 460 West 34th Street – 17th floor
> > New York, NY 10001
> >
> > (b) (6) (direct)

(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Wed, Mar 21, 2018 at 5:42 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Here's the letter you need. -Tonie
> ---------- Forwarded message ----------
> From: **Schroeder, Rebecca** <rschroeder@usgs.gov>
> Date: Wed, Mar 21, 2018 at 4:09 PM
> Subject: Re: letter for Jonathan to sign
> To: "Rocke, Tonie" <trocke@usgs.gov>
>
> Here you go.
>
> Becky Schroeder
> Executive Assistant to the Director's Office
> USGS National Wildlife Health Center
> 6006 Schroeder Rd.
> Madison, WI 53711
> Phone: (608) 270-2402
> Email: RSchroeder@usgs.gov
>
> On Wed, Mar 21, 2018 at 1:07 PM, Rocke, Tonie <trocke@usgs.gov> wrote:
>
> > Hi Rebecca: Can you put this draft letter on our letterhead, add Jonathan's credentials as he likes to use, and get his signature? If you send the signed letter back to me, I'll pass it on. Thanks much! -Tonie
> >
> > --
> > Tonie E. Rocke
> > USGS National Wildlife Health Center
> > 6006 Schroeder Rd.
> > Madison, WI 53711
> > 608-270-2451
> > trocke@usgs.gov
>
> --
> Tonie E. Rocke
> USGS National Wildlife Health Center
> 6006 Schroeder Rd.
> Madison, WI 53711
> 608-270-2451
> trocke@usgs.gov

>

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

From: Rocke, Tonie <trocke@usgs.gov>
Sent: Tuesday, March 20, 2018 6:18 PM
To: Jerome.Unidad@parc.com
Subject: Re: Task 7 Text, PARC inclusion

Thanks Jerome. I'll take a look. Still haven't seen Peter's updates yet so I imagine this will change at least slightly. Best -Tonie

On Tue, Mar 20, 2018 at 7:04 PM, <Jerome.Unidad@parc.com> wrote:

> Tonie,
>
> In reference to my previous email, I've made the changes highlighted in cyan/light blue to include PARC. Let me know if this is sufficient. If you feel like we need further data/figures regarding the aerosol technology, for example, we could also include something like Fig. 1 from our white paper. But I don't think it's necessary – let me know your thoughts on this.
>
> Thanks,
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

From: Jerome.Unidad@parc.com
Sent: Tuesday, March 20, 2018 5:05 PM
To: trocke@usgs.gov
Subject: Task 7 Text, PARC inclusion
Attachments: PREEMPT TR task 7 first draft_JU.docx; PARC_whitepaper_Biotech_v3_final.pdf

Tonie,

In reference to my previous email, I've made the changes highlighted in cyan/light blue to include PARC. Let me know if this is sufficient. If you feel like we need further data/figures regarding the aerosol technology, for example, we could also include something like Fig. 1 from our white paper. But I don't think it's necessary – let me know your thoughts on this.

Thanks,

Jerome

Jerome Unidad, PhD
Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory
PARC, A Xerox Company

Task 7: Develop and assess delivery methods to bats for immune boosting and priming molecules

Description and execution: While work is proceeding to identify and optimize immunomodulating agents to manage SARS-Coronaviruses, we will concurrently develop and test mediums, routes, and methods of delivery to large colonies of bats. Several different approaches or combinations of approaches will be assessed to determine the most feasible and simplest method of delivery that achieves high uptake by bats, is safe for humans as well as target and non-target species, and minimizes disturbance to the colony. Sticky edible gels or pastes that bats groom from themselves and each other have been used previously to deliver pharmaceuticals to bats orally and are currently being tested as a medium for delivery of vaccines against rabies and other diseases in wild bats (see preliminary data). These may also be useful for delivering immune modulators and recombinant SARSr-CoV spike proteins to *Rhinolophus* bats, but may need to be combined with viral vectors (like poxvirus or adenovirus) or nanoparticles/nanoemulsions that enhance uptake through mucous membranes or transdermally after topical application.

Poxviruses in particular have been demonstrated to be effective viral vectors for delivering vaccines to wildlife (Slate et al., 2009) Freuling et al., 2013; Rocke et al., 2017). Recent laboratory studies in bats have shown that poxviruses can replicate safely at high levels in bats after oronasal administration (Stading et al., 2016)m and poxvirus vectored vaccines are immunogenic, protecting bats from rabies challenge (Stading et al 2017; see preliminary data). Poxviruses are highly safe, having been tested in a wide variety of wild and domestic animals, they allow for large inserts of foreign DNA, and they have a proven record of success. Poxviruses are good candidates for this project, but we will also consider others.

In addition to viral vectors, we will also consider methods to achieve transcutaneous delivery of the immune boosting proteins without the use of live agents. Recent advances in methods to achieve transdermal or transcutaneous delivery of drugs and vaccines have been reported. (Roberts et al., 2017). However, a major impediment to this route of vaccination is the stratum corneum, the outermost barrier layer of the skin that protects underlying layers from infection and damage. Numerous approaches have relied on mechanical methods to compromise the stratum corneum to allow the drug or vaccine to penetrate into the skin (Roberts et al., 2017). Innovations in nanotechnology show promise in being able to deliver drugs and vaccines into the deeper layers of the skin without the need for damage to the stratum corneum (Mishra et al., 2013), an important consideration. Dendritic cells and Langerhans cells, antigen-presenting cells which reside in the dermis and epidermis, can take up these transdermally delivered proteins and generate an immune response. We are currently testing poly lactic-co-glycolic acid (PLGA) as a nanoparticle to encapsulate rabies glycoprotein as a method of transcutaneous delivery of vaccine to bats. PLGA has been used previously to deliver both toll-like receptor agonists and antigens simultaneously to mice (Ebrahimian, 2017). This and other products (outlined above in Task ?) could potentially be useful with SARSr-CoV glycoproteins. Adjuvants can also be incorporated into nanoemulsions and nanoparticles to amplify the natural immune response to the vaccine antigens (Karande and Mitragotri, 2010). With SARS-CoV spike proteins, the adjuvant Matrix M1

(Isconova, Sweden) has been shown to significantly enhance the immune response in mice (Coleman et al. 2014)

In collaboration with Dr. Baric and others, we will determine the most likely immunomodulating formulations based on the results of TA2, previous animal studies and other available data and then use both laboratory and field studies to assess and optimize delivery vehicles and methods for wild bats. To reduce costs, initial studies will be conducted with locally acquired insectivorous bats (*Eptesicus fuscus*--big brown bats). We have successfully maintained and housed big brown bats and other insectivorous species for several experiments at our facility previously (Stading et al., 2016, 2017). We will treat bats via topical application with various test formulations that include the biomarker Rhodamine B (RB), co-house them with untreated bats, and monitor transfer between bats by collecting hair and whiskers for biomarker analysis. Rhodamine B is detectable within the hair of animals within 24 hours of consumption using a fluorescence microscope, and we have considerable experience using this biomarker for similar studies (see preliminary data).

Once we have confirmed uptake in laboratory studies, we will then assess mass delivery methods in local caves and hibernacula (using biomarker-labeled mediums but without immunomodulatory substances). In collaboration with Dr. Jerome Unidad of Palo Alto Research Center, we will explore the use of innovative aerosol technology that could be used in cave settings in the form of a field-deployable spray device triggered by timers and movement detectors at critical cave entry points. The PARC technology called Filament Extension Atomization (FEA) can spray fluids with a wide-range of viscosities ranging from 1mPa-s to 100Pa-s using a roll-to-roll misting process (further details in the PARC website). This will make it compatible with all the fluid formulations mentioned earlier including the immunomodulating formulations from TA2, gels and creams for topical delivery and Poxvirus formulations, making it a universal platform for inoculating the bats. Within one week of application, bats will be trapped at the cave entrace using mist nets or Harp traps and hair will be collected to assess the rate of uptake via biomarker analysis. The bats will be released immediately afterward. The procedures will be tested at several different locations as it will likely take some manipulation to determine appropriate dosages for maximum uptake. After we have determined the most optimal approaches for mass delivery, we will then test them on wild bats in our three cave sites in Yunnan Province. Again, biomarker will be used to assess rates of uptake and this data can then be used in modeling studies to help determine the optimal rates of application of immunomodulating agents. Biomarker studies can also be used to assess uptake by non-target species, an important consideration in evaluating safety. Fieldwork will be conducted in collaboration with Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance).

Preliminary Data: Rocke and colleagues have developed oral vaccines and delivery methods to manage disease in free-ranging wildlife for many years, including a sylvatic plague vaccine for prairie dogs (Rocke et al., 2017), and more recently, vaccines against rabies (Stading et al., 2017) and white-nose syndrome for bats (Rocke, unpublished data). In addition to developing, testing and registering vaccines for experimental field use, vaccine delivery methods and uptake by the target species were optimized using biomarker studies prior to deployment; biomarker studies were also used to assess uptake

and safety in non-target hosts (Tripp et al., 2015). A similar approach will be used to develop, test and optimize delivery methods to *Rhinolophus* bats in SE Asia.

To manage plague caused by *Yersinia pestis* in prairie dogs, a raccoon poxvirus vectored vaccine expressing plague antigens was incorporated into a peanut-butter flavored bait matrix. Rhodamine B (RB), a biomarker that dyes hair, whiskers and feces and is visible within 24 hours of consumption by animals, was included in the baits in order to assess uptake by both target and non-target species (Figure 1). When viewed under a UV microscope at a specific wavelength, the biomarker is visible until the hair grows out (approximately 50 days in prairie dogs). Biomarker studies were initially used to assess palatability and acceptance of the bait matrix by wild prairie dogs (Tripp et al., 2014) and also used to assess bait ingestion by non-target rodents (Tripp et al., 2015). After safety was confirmed in non-targets and with the approval of USDA Center for Veterinary Biologics, a large field trial was conducted over a 3-year period that demonstrated vaccine effectiveness in four species of prairie dogs in seven western states (Rocke et al., 2017). Using biomarker analysis, we then assessed site- and individual host-level factors related to bait consumption in prairie dogs to determine those most related to increased bait consumption, including age, weight, and the availability of green vegetation. Identifying the factors that maximize the likelihood of expedient bait uptake by targeted individuals is important for developing strategies to optimize vaccine effectiveness. This will also be important in developing disease management strategies for bats.

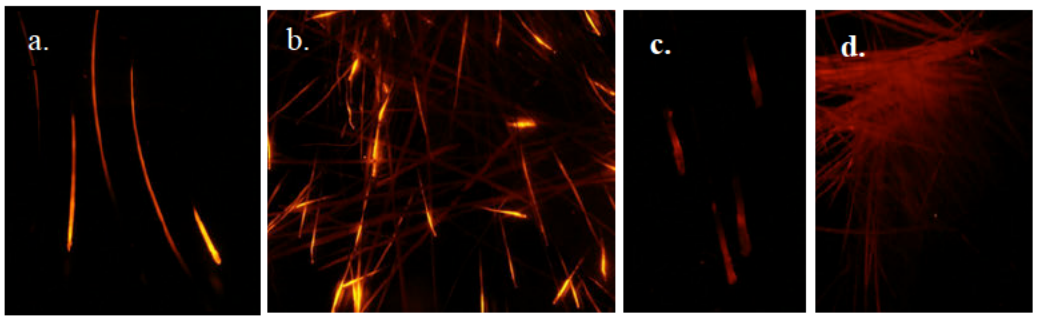

Figure 1. Prairie dog hair and whisker samples viewed under fluorescence microscope (excitation wavelength: 540 nm, emission wavelength: 625 nm) to determine uptake of baits containing Rhodamine B. a) whiskers positive for RB uptake 20 days after bait distribution, b) hair sample positive for RB uptake 16 days after bait distribution, c and d) whiskers and hair negative for RB uptake 20 days after bait distribution (note natural dull fluorescence).

In recent years, our research team has been developing and testing vaccines and delivery methods for use in free-ranging bats. First we tested two commonly used viral vectors, modified vaccinia Ankara (MVA) and raccoon poxvirus (RCN), for their safety and replication in bats using in vivo biophotonic imaging. (Stading et al. 2017). RCN replicated to higher levels in bats than MVA, even via the oral route, and was found to be highly safe for bats (Figure 2). We then used raccoon poxvirus as a viral vector to express a novel rabies glycoprotein (mosaic or MoG) and tested the protective efficacy of this construct in bats after both oronasal and topical administration (Stading et al 2017).

Both methods of application were successful, protecting nearly all of the immunized and challenged bats (Figure 3), work is now progressing to develop methods of vaccine delivery to vampire bats, one of the primary reservoirs of rabies for both humans and animals, primarily cattle, in several Latin American countries. We are also using a similar approach to develop vaccines for white-nose syndrome in bats, a devastating disease that has killed millions of insectivorous bats in North America.

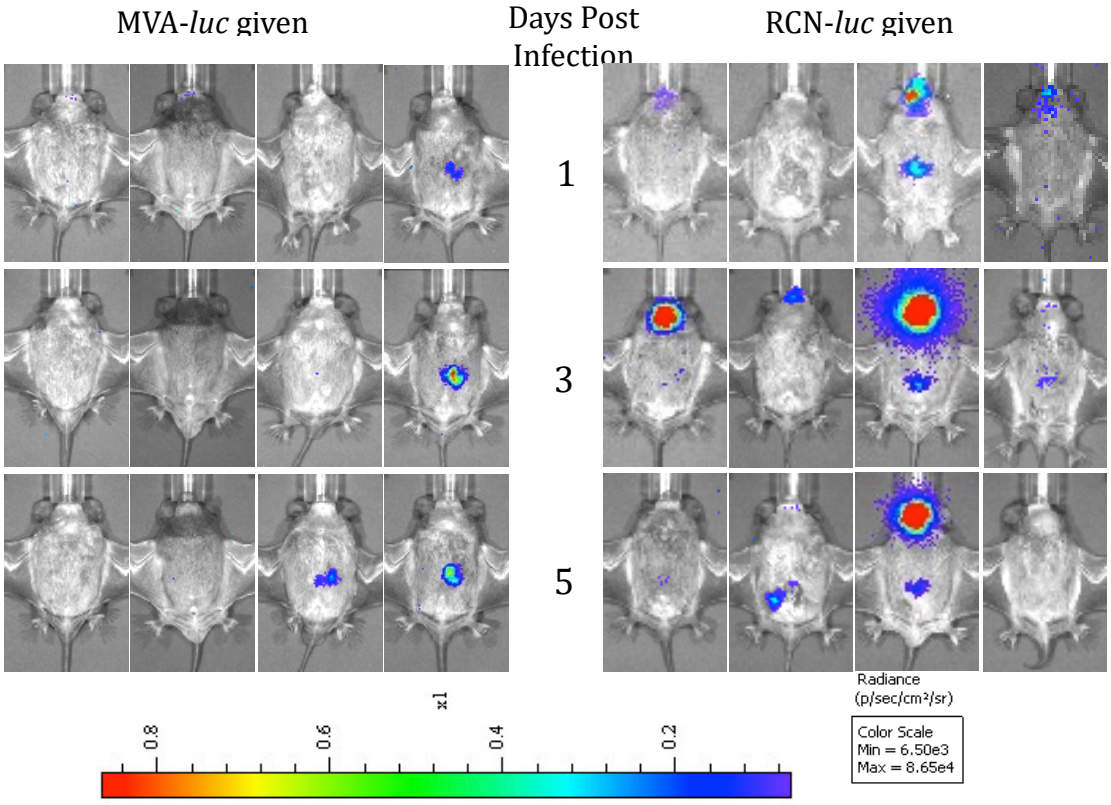

Figure 2. Luminescence, indicative of viral replication of modified vaccinia Ankara (MVA) and raccoon poxvirus RCN) in *Tadarida brasiliensis* on days 1, 3 and 5 post-inoculation via the oronasal route.

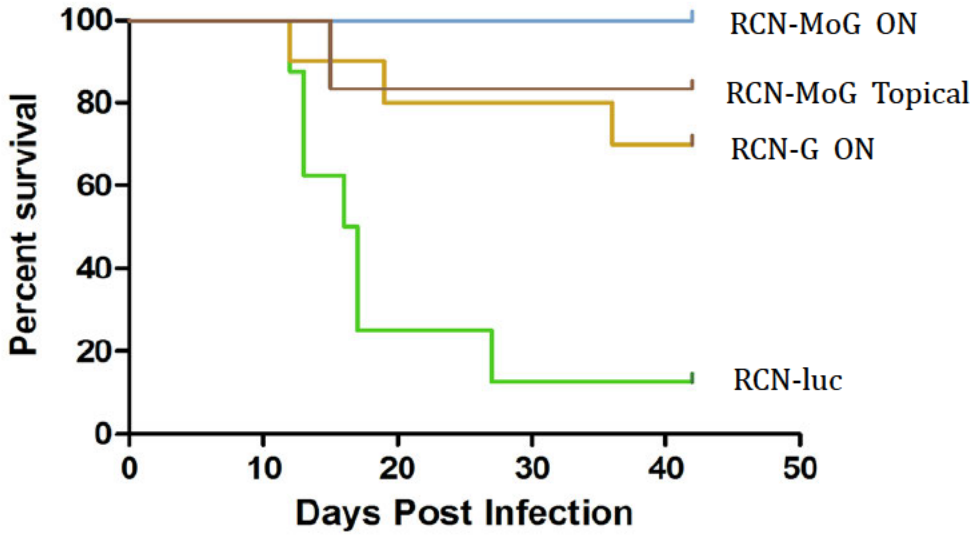

Figure 3. Results of vaccine efficacy and rabies challenge trials in *Epstesicus fuscus* immunized with raccoon poxvirus expressing a mosaic G protein (RCN-MoG) either oronasally (ON) or topically in comparison to RCN expressing typical G protein and RCN expressing luciferase (a negative control).

For bats a different approach is required for vaccine delivery, as in general, they are not attracted to baits. Bats, especially vampire bats, are known to practice self and mutual grooming at a high rate, and this behavior has been exploited to cull vampire bats using poisons like warfarin. The poison is applied topically to a number of bats that are released. When they return to their roost, the poison is transferred to roost-mates by contact and mutual grooming. We are exploiting this same behavior for vaccine application. Preliminary biomarker studies (without vaccine) are being conducted in vampire bats in both Mexico and Peru and also in insectivorous bats in Wisconsin. In a pilot study in Peru, we treated 50 bats from a single cave with RB-labelled glycerin jelly. Based on capture-recapture data, we estimated the population at ~200 bats, so ~25% of bats were initially marked. Upon trapping of this population a few days later, 64 bats were captured, including 19 originally marked bats (Table 1 – could be made into a figure instead). Hair was collected and examined for RB marking under a fluorescence microscope. All treated bats were positive for RB marking in addition to 39% of newly captured bats, indicating a rate of transfer of about 1.3 bats for every bat marked. Additional trials have been conducted, with transfer rates of up to 2.8 bats for every bat treated achieved at least once. These trials are being analyzed to assess factors associated with rates of transfer, e.g. sex and age of initially treated bats, time of day, etc. This data is then being used to model the rate of vaccination and impact on rabies transmission with different rates of application, prior to actual deployment of vaccine in the field.

Table 1. Marking of vampire bats a few days after application of glycerin jelly containing Rhodamine B.

	Number captured	Positive	Negative	Inconclusive	% positive (w/o inc)
All bats	64	34	25	5	58
Recaptured marked bats	19	18	0	1	100
New bat captures	45	16	25	4	39

For insectivorous bats, we are trying other approaches. Instead of hand applying the jelly to bats, we applied RB marked glycerin jelly to the entry of bat houses used by little brown bats (*Myotis lucifugus*). The bats became covered as they entered the houses and then consumed the material during self and mutual grooming. One week later, bats were trapped at the houses to determine the rate of uptake. Of 29 bats trapped one week post-application, 59% (17) were positive for biomarker indicating they had eaten the jelly. Thus, with additional optimization, application of vaccine to bat houses or other structures (small cave entrances) could also be a viable method of delivery. In addition, we are considering different spray applications directly to roosting bats in caves and through motion-sensing sprayers at cave entrances. Whatever the means of application, effective treatment relies on ingestion by bats, and that is easily confirmed with the use of the biomarker, RB.

Organization leading task: USGS National Wildlife Health Center

Progress Metrics: Not sure exactly what format to use here

Deliverable(s):
Medium and methods to deliver immunomodulatory agents to bats.
Data on uptake in insectivorous bats.
Reports, manuscripts, presentations.

Coleman CM, Liu YV, Mu H, Taylor JK, Massare M, Flyer DC, Smith GE, Frieman MB. 2014. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine 32:3169-3174.

Ebrahimian M, Hashemi M, Maleki M, Hashemitabar G, Abnous K, Ramezani M, Haghparast A. 2017. Co-delivery of dual toll-like receptor agaonists and antigen in poly(lactic-co-glycolic) acid/polyethylenimine cationic hybrid nanoparticles promote efficient in vivo immune responses. Front Immunol 8:1077.

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Karande P, Mitragotri S. 2010. Transcutaneous immunization: an overview of advantages, disease targets, vaccines, and delivery technologies. Annu Rev Chem Biomol Eng 1:175-201.

Mishra DK, Dhote V, Mishra PK. 2013. Transdermal immunization: biological framework and translational perspectives. Expert Opin Drug Deliv 10:183-200.

PARC Website: Advanced Manufacturing and Deposition Systems Group – https://www.parc.com/services/focus-area/amds/

Roberts MS, Mohammed Y, Pastore MN, Namjoshi S, Yousef S, Alinaghi A, Haridass IN, Abd E, Leite-Silva VR, Benson HAE, Grice JE. 2017. Topical and cutaneous delivery using nanosystems. J Control Release 247:86-105.

Rocke TE, Tripp DW, Russell RE, Abbott RC, Richgels KLD, Matchett MR, Biggins DE, Griebel R, Schroeder G, Grassel SM, Pipkin DR, Cordova J, Kavalunas A, Maxfield B, Boulerice J, Miller MW. 2017. Sylvatic plague vaccine partially protects prairie dogs (*Cynomys* spp.) in field trials. EcoHealth DOI: 10.1007/s10393-017-1253-x.

Slate D, Algeo TP, Nelson KM, Chipman RB, Donovan D, Blanton JD, Niezgoda M, Rupprecht CE (2009) Oral rabies vaccination in North America: opportunities, complexities, and challenges. PLoS Neglected Tropical Diseases 22 3(12):e549.doi:10.1371/journal.pntd.0000549

Stading BR, Osorio JE, Velasco-Villa A, Smotherman M, Kingstad-Bakke B, Rocke TE. Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). Vaccine. 2016;34: 5352–5358. doi:10.1016/j.vaccine.2016.08.088

Stading B, Ellison JA, Carson WC, Panayampalli SS, Rocke TE, Osorio JE. Protection of bats (*Eptesicus fuscus*) against rabies following topical or oronasal exporue to a recombinant raccoon poxvirus vaccine. PLoS Negl Trop Dis 11:e0005958.

Tripp DW, Rocke TE, Streich SP, Brown NL, Fernandez JR-R, Miller MW. 2014. Season and application rates affect vaccine bait consumption by prairie dogs in Colorado and Utah, USA. J Wildlife Dis 20:

Tripp DW, Rocke TE, Streich SP, Abbott RC, Osorio JE, Miller MW. 2015. Apparent field safety of a raccoon poxvirus-vectored plague vaccine in free-ranging prairie dogs, Colorado, USA. J Wildlife Dis 51:

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Innovative Solutions for Biotechnology @ PARC

Leveraging on our long history in fluid and particle manipulation and roll-to-roll processes for printing applications, the PARC hardware laboratories are currently developing innovative device solutions with potential applications in biomedicine and biotechnology at-large.

Filament Extension Atomizer (FEA)

PARC is developing a novel spray technology called the **Filament Extension Atomizer (FEA)** that can generate aerosol from fluids that are notoriously difficult to aerosolize due to the inherent *viscosity limit* with most conventional spray methods. FEA can spray fluids of a wide range of viscosities: from 1 mPa-s (the viscosity of water) up to 1000 Pa-s (the viscosity of peanut butter) – this range includes fluids or dispersions with significant (bio)macromolecular content. This macromolecular content (long chain polymers) often impart an additional resistance to aerosol/spray generation due to *strain hardening* or the increase of fluid viscosity as a function of extension.

To generate aerosol from such *strain hardening* fluids, FEA harnesses a well-known *elasto-capillary instability* that generates beads-on-a-string formation (Fig. 1A) when a fluid is held in extension. As the filaments are sufficiently thinned out, droplet break-up occurs and generates free droplets (aerosol). The FEA technology implements similar mechanics in a roll-to-roll process (Fig. 1B) to massively parallelize the filament formation and break-up and continuously generate droplets. To date, we have applied this on multiple viscoelastic fluids which include polymer solutions, polymer melts, particle dispersions and other complex systems with biomolecules (Figs. 1C-E).

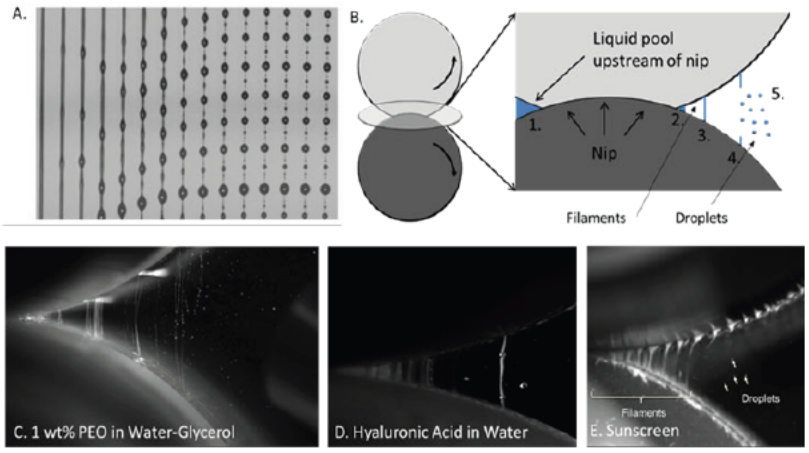
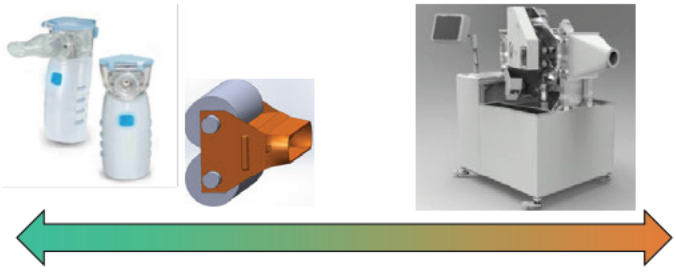


Fig. 1 – A. Beads-on-a-string structures in a viscoelastic fluid in extension (McKinley and Olivera 2005) B. Multiple beads-on-a-string formations in counter-rotating rollers (FEA) FEA spraying of PEO solution (C.) hyaluronic acid (D.) and sunscreen (E.)



Consumer Scale
- Portable devices
- Small rollers (down to 10mm)
- Low throughput
- Precision fluid delivery (e.g. bioactive doses)
- Smart, connected devices

Industrial Scale
- Large rollers (up to 100mm)
- High throughput
- Unit operation in a manufacturing line
- Particle creation (e.g. spray drying), large area coatings

Fig. 2 – FEA Technology at Different Scales and Applications

The FEA technology is inherently scalable – it can work with small rollers for consumer scale applications, tailored for precision fluid dispensing, or with large rollers for industrial scale, high throughput aerosol generation for coatings or for powder production via spray drying. Since the FEA technology applies to wide range of fluids of virtually any viscosity or composition, it allows nearly formulation-independent fluid delivery. This can have a huge impact in biomedicine and biotechnology at-large: fluids can be *formulated for bio-efficacy* and not for delivery with no limits on bioactive loading, even with high viscosity biomacromolecules that are notoriously hard to deliver in a controlled, reliable manner. FEA can also allow the creation of specialized particles for drug delivery with a wide range of material set and control over the particle morphology.

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Technical Contact:
Jerome Unidad (Jerome.Unidad@parc.com), Member of Research Staff

A global center for commercial innovation, PARC, a Xerox company, works closely with enterprises, entrepreneurs, government program partners and other clients to discover, develop, and deliver new business opportunities. PARC was incorporated in 2002 as a wholly owned subsidiary of Xerox Corporation (NYSE: XRX).

Re: Task 7 Text, PARC inclusion

Luke Hamel <hamel@ecohealthalliance.org>

Wed 3/21/2018 10:19 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Daszak Peter <daszak@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>

Thank you for sending this, Tonie.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Wed, Mar 21, 2018 at 6:04 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> ---------- Forwarded message ----------
> From: <Jerome.Unidad@parc.com>
> Date: Wed, Mar 21, 2018 at 1:00 PM
> Subject: RE: Task 7 Text, PARC inclusion
> To: trocke@usgs.gov
>
> Here's the version with an added figure. This should be ok, I'll withhold further edits until we get the full technical volume.
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> Thanks,
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Tuesday, March 20, 2018 6:39 PM
To: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
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Tonie E. Rocke

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To: Daszak Peter; Luke Hamel; Anna Willoughby
Subject: Fwd: Task 7 Text, PARC inclusion
Attachments: PREEMPT TR task 7 first draft_JU_with_figure.docx

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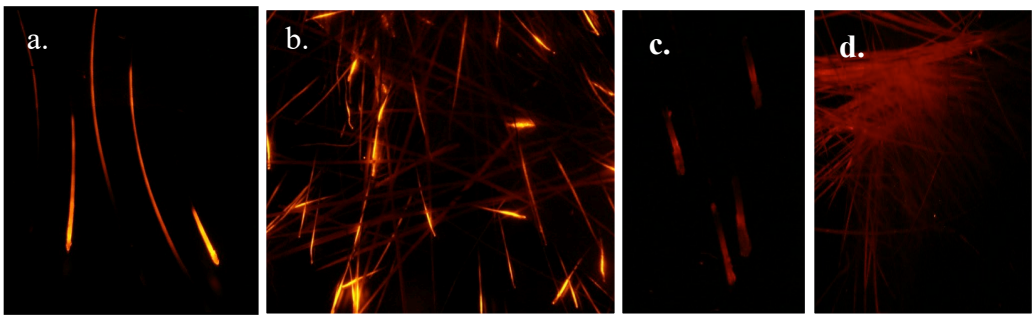

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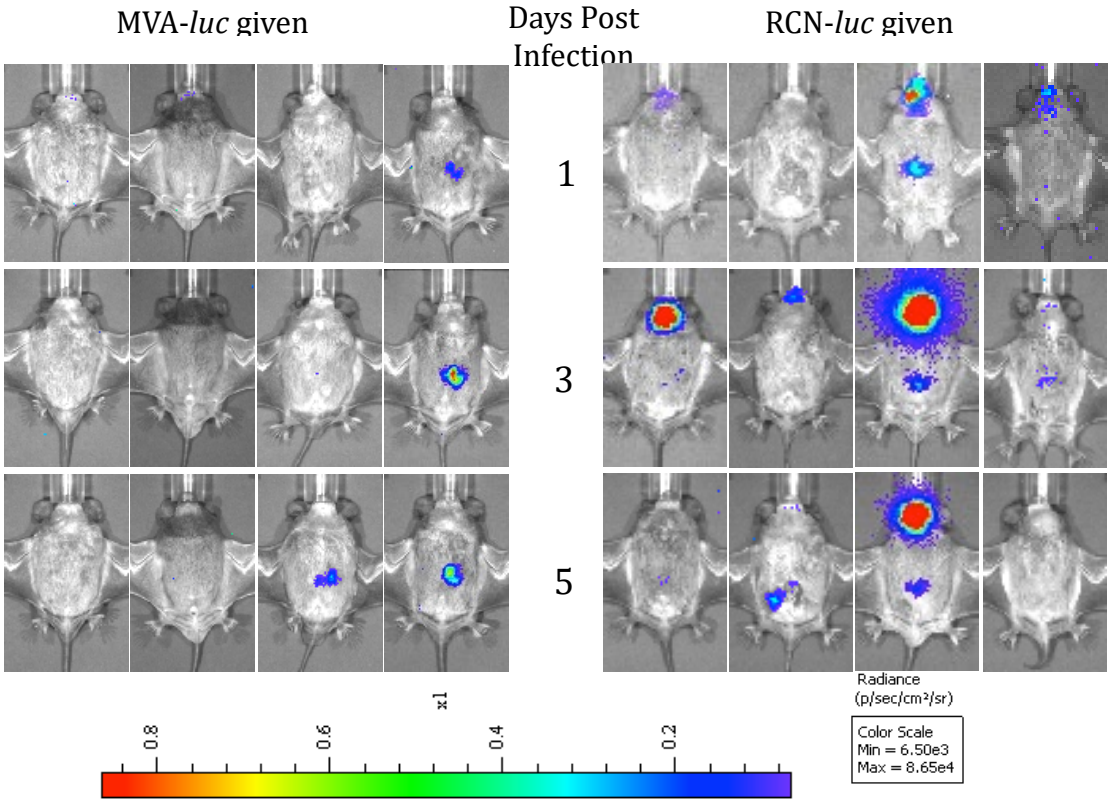

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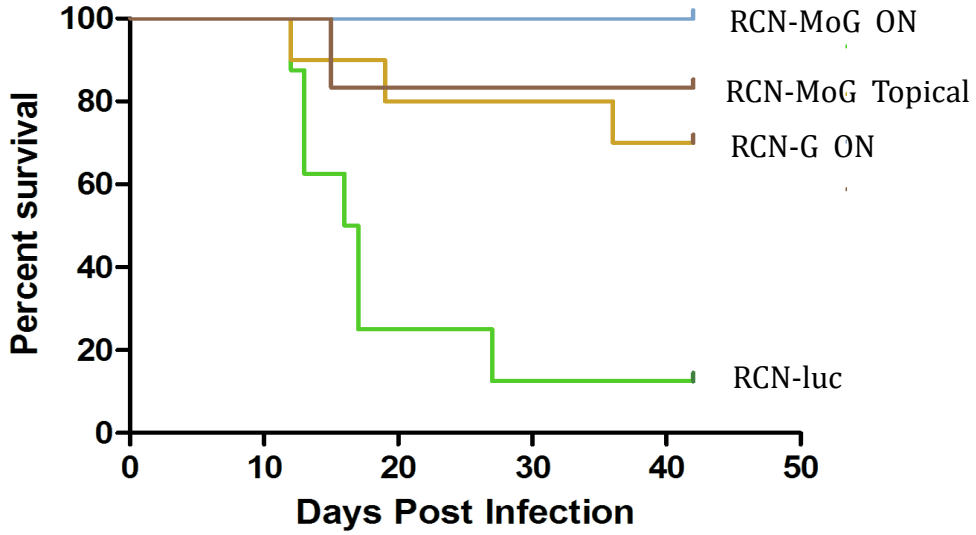

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For bats a different approach is required for vaccine delivery, as in general, they are not attracted to baits. Bats, especially vampire bats, are known to practice self and mutual grooming at a high rate, and this behavior has been exploited to cull vampire bats using poisons like warfarin. The poison is applied topically to a number of bats that are released. When they return to their roost, the poison is transferred to roost-mates by contact and mutual grooming. We are exploiting this same behavior for vaccine application. Preliminary biomarker studies (without vaccine) are being conducted in vampire bats in both Mexico and Peru and also in insectivorous bats in Wisconsin. In a pilot study in Peru, we treated 50 bats from a single cave with RB-labelled glycerin jelly. Based on capture-recapture data, we estimated the population at ~200 bats, so ~25% of bats were initially marked. Upon trapping of this population a few days later, 64 bats were captured, including 19 originally marked bats (Table 1 – could be made into a figure instead). Hair was collected and examined for RB marking under a fluorescence microscope. All treated bats were positive for RB marking in addition to 39% of newly captured bats, indicating a rate of transfer of about 1.3 bats for every bat marked. Additional trials have been conducted, with transfer rates of up to 2.8 bats for every bat treated achieved at least once. These trials are being analyzed to assess factors associated with rates of transfer, e.g. sex and age of initially treated bats, time of day, etc. This data is then being used to model the rate of vaccination and impact on rabies transmission with different rates of application, prior to actual deployment of vaccine in the field.

Table 1. Marking of vampire bats a few days after application of glycerin jelly containing Rhodamine B.

	Number captured	Positive	Negative	Inconclusive	% positive (w/o inc)
All bats	64	34	25	5	58
Recaptured marked bats	19	18	0	1	100
New bat captures	45	16	25	4	39

For insectivorous bats, we are trying other approaches. Instead of hand applying the jelly to bats, we applied RB marked glycerin jelly to the entry of bat houses used by little brown bats (*Myotis lucifugus*). The bats became covered as they entered the houses and then consumed the material during self and mutual grooming. One week later, bats were trapped at the houses to determine the rate of uptake. Of 29 bats trapped one week post-application, 59% (17) were positive for biomarker indicating they had eaten the jelly. Thus, with additional optimization, application of vaccine to bat houses or other structures (small cave entrances) could also be a viable method of delivery. In addition, we are considering different spray applications directly to roosting bats in caves and through motion-sensing sprayers at cave entrances. Whatever the means of application, effective treatment relies on ingestion by bats, and that is easily confirmed with the use of the biomarker, RB.

PARC will develop the FEA aerosol technology wide-scale inoculation of bats in PRE-EMPT. Fig.4 shows the basic principle of the technology and the resulting spray from representative fluids (aqueous polymer solutions, consumer formulations). FEA technology can be used for the full range of fluids of interest to the program including gels and creams for topical application and aqueous/non-aqueous vaccine formulations. Further details can be found in the PARC website (see references).

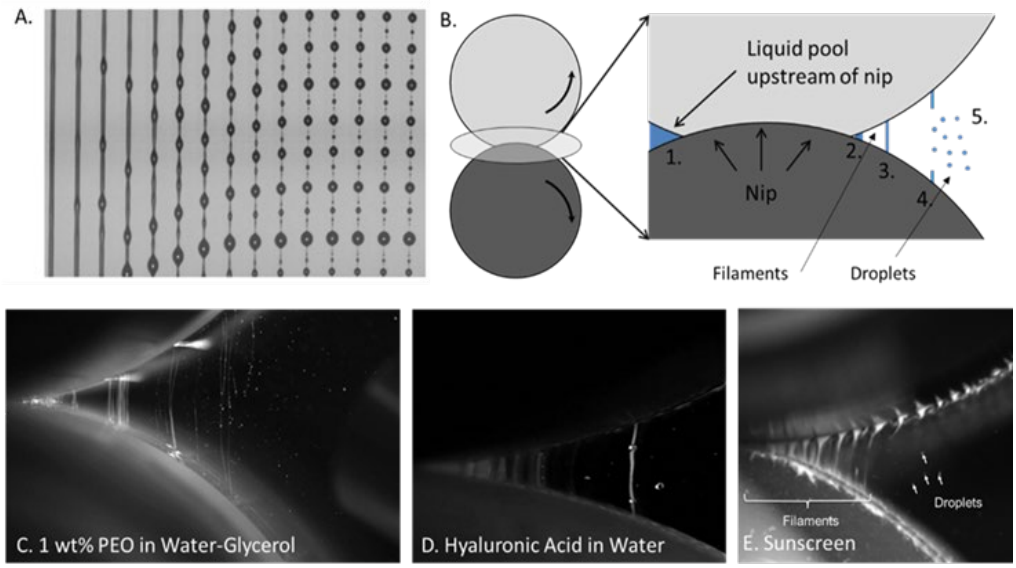

Figure 4. FEA technology: A. Beads-on-a-string formation in viscoelastic fluids in extension (Oliveira and McKinley, 2005), B. Roll-to-roll parallelization of filament formation and break-up in FEA, C.-E. Examples of fluids sprayed with FEA including polyethylene oxide in water-glycerol (C.), hyaluronic acid in water (D.) and sunscreen (E.)

Organization leading task: USGS National Wildlife Health Center
Participating organizations: Palo Alto Research Center (PARC)

Progress Metrics: Not sure exactly what format to use here

Deliverable(s):
Medium and methods to deliver immunomodulatory agents to bats.
Data on uptake in insectivorous bats.
Reports, manuscripts, presentations.

Coleman CM, Liu YV, Mu H, Taylor JK, Massare M, Flyer DC, Smith GE, Frieman MB. 2014. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine 32:3169-3174.

Ebrahimian M, Hashemi M, Maleki M, Hashemitabar G, Abnous K, Ramezani M, Haghparast A. 2017. Co-delivery of dual toll-like receptor agaonists and antigen in poly(lactic-co-glycolic) acid/polyethylenimine cationic hybrid nanoparticles promote efficient in vivo immune responses. Front Immunol 8:1077.

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PARC Website: Advanced Manufacturing and Deposition Systems Group – https://www.parc.com/services/focus-area/amds/

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Stading B, Ellison JA, Carson WC, Panayampalli SS, Rocke TE, Osorio JE. Protection of bats (*Eptesicus fuscus*) against rabies following topical or oronasal exporue to a recombinant raccoon poxvirus vaccine. PLoS Negl Trop Dis 11:e0005958.

Tripp DW, Rocke TE, Streich SP, Brown NL, Fernandez JR-R, Miller MW. 2014. Season and application rates affect vaccine bait consumption by prairie dogs in Colorado and Utah, USA. J Wildlife Dis 20:

Tripp DW, Rocke TE, Streich SP, Abbott RC, Osorio JE, Miller MW. 2015. Apparent field safety of a raccoon poxvirus-vectored plague vaccine in free-ranging prairie dogs, Colorado, USA. J Wildlife Dis 51:

From: Rocke, Tonie <trocke@usgs.gov>
Sent: Wednesday, March 21, 2018 11:08 AM
To: Jerome.Unidad@parc.com
Subject: Re: Task 7 Text, PARC inclusion

OK looks good to me. -Tonie

On Wed, Mar 21, 2018 at 1:00 PM, <Jerome.Unidad@parc.com> wrote:

> Here's the version with an added figure. This should be ok, I'll withhold further edits until we get the full technical volume.
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> ---
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> Jerome Unidad, PhD
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>
> PARC, A Xerox Company
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> **From:** Rocke, Tonie [mailto:trocke@usgs.gov]
> **Sent:** Tuesday, March 20, 2018 6:39 PM
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To: trocke@usgs.gov
Subject: RE: Task 7 Text, PARC inclusion
Attachments: PREEMPT TR task 7 first draft_JU_with_figure.docx

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Task 7: Develop and assess delivery methods to bats for immune boosting and priming molecules

Description and execution: While work is proceeding to identify and optimize immunomodulating agents to manage SARS-Coronaviruses, we will concurrently develop and test mediums, routes, and methods of delivery to large colonies of bats. Several different approaches or combinations of approaches will be assessed to determine the most feasible and simplest method of delivery that achieves high uptake by bats, is safe for humans as well as target and non-target species, and minimizes disturbance to the colony. Sticky edible gels or pastes that bats groom from themselves and each other have been used previously to deliver pharmaceuticals to bats orally and are currently being tested as a medium for delivery of vaccines against rabies and other diseases in wild bats (see preliminary data). These may also be useful for delivering immune modulators and recombinant SARSr-CoV spike proteins to *Rhinolophus* bats, but may need to be combined with viral vectors (like poxvirus or adenovirus) or nanoparticles/nanoemulsions that enhance uptake through mucous membranes or transdermally after topical application.

Poxviruses in particular have been demonstrated to be effective viral vectors for delivering vaccines to wildlife (Slate et al., 2009) Freuling et al., 2013; Rocke et al., 2017). Recent laboratory studies in bats have shown that poxviruses can replicate safely at high levels in bats after oronasal administration (Stading et al., 2016)m and poxvirus vectored vaccines are immunogenic, protecting bats from rabies challenge (Stading et al 2017; see preliminary data). Poxviruses are highly safe, having been tested in a wide variety of wild and domestic animals, they allow for large inserts of foreign DNA, and they have a proven record of success. Poxviruses are good candidates for this project, but we will also consider others.

In addition to viral vectors, we will also consider methods to achieve transcutaneous delivery of the immune boosting proteins without the use of live agents. Recent advances in methods to achieve transdermal or transcutaneous delivery of drugs and vaccines have been reported. (Roberts et al., 2017). However, a major impediment to this route of vaccination is the stratum corneum, the outermost barrier layer of the skin that protects underlying layers from infection and damage. Numerous approaches have relied on mechanical methods to compromise the stratum corneum to allow the drug or vaccine to penetrate into the skin (Roberts et al., 2017). Innovations in nanotechnology show promise in being able to deliver drugs and vaccines into the deeper layers of the skin without the need for damage to the stratum corneum (Mishra et al., 2013), an important consideration. Dendritic cells and Langerhans cells, antigen-presenting cells which reside in the dermis and epidermis, can take up these transdermally delivered proteins and generate an immune response. We are currently testing poly lactic-co-glycolic acid (PLGA) as a nanoparticle to encapsulate rabies glycoprotein as a method of transcutaneous delivery of vaccine to bats. PLGA has been used previously to deliver both toll-like receptor agonists and antigens simultaneously to mice (Ebrahimian, 2017). This and other products (outlined above in Task ?) could potentially be useful with SARSr-CoV glycoproteins. Adjuvants can also be incorporated into nanoemulsions and nanoparticles to amplify the natural immune response to the vaccine antigens (Karande and Mitragotri, 2010). With SARS-CoV spike proteins, the adjuvant Matrix M1

(Isconova, Sweden) has been shown to significantly enhance the immune response in mice (Coleman et al. 2014)

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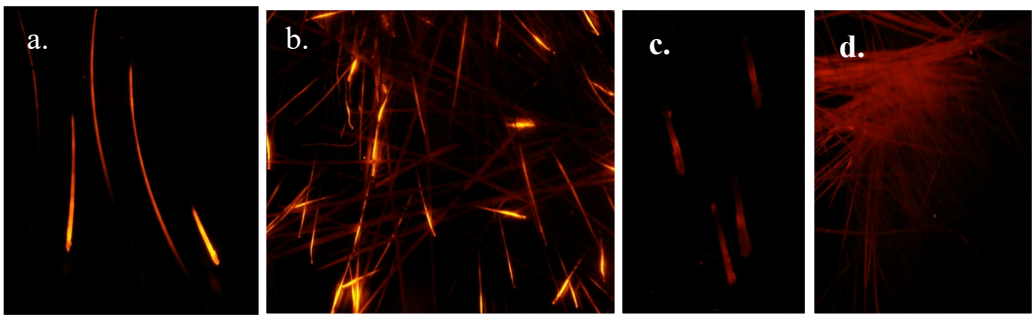

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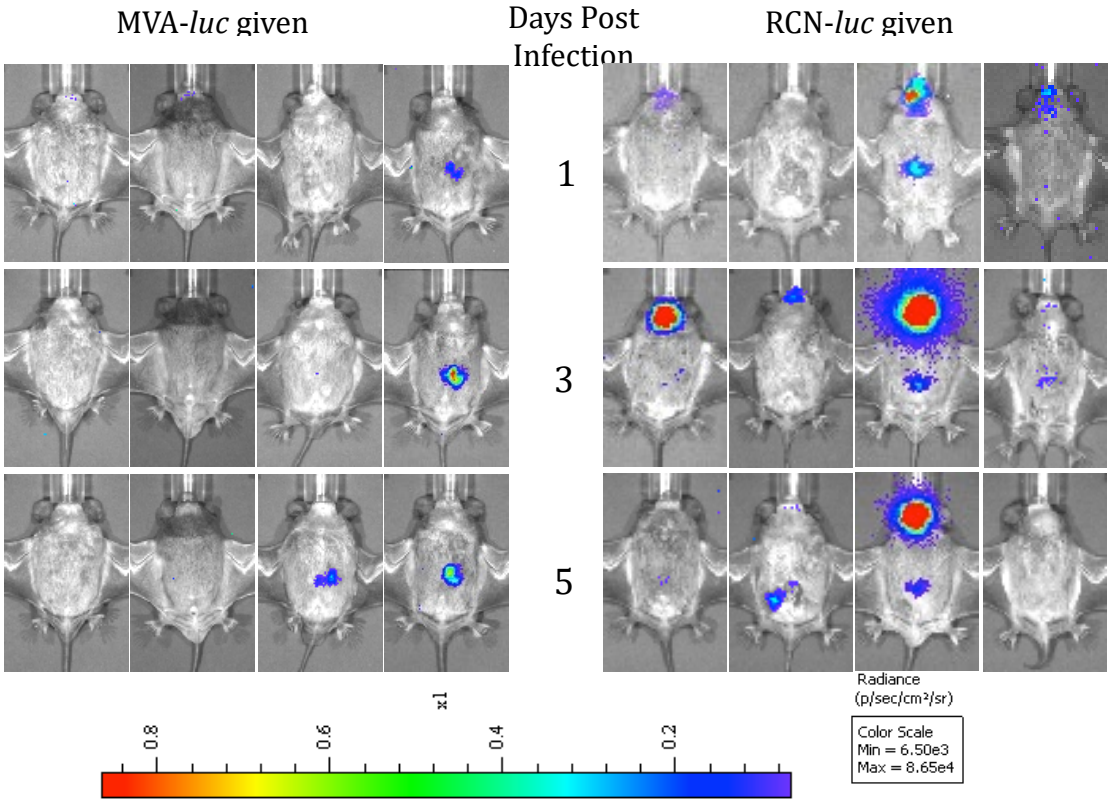

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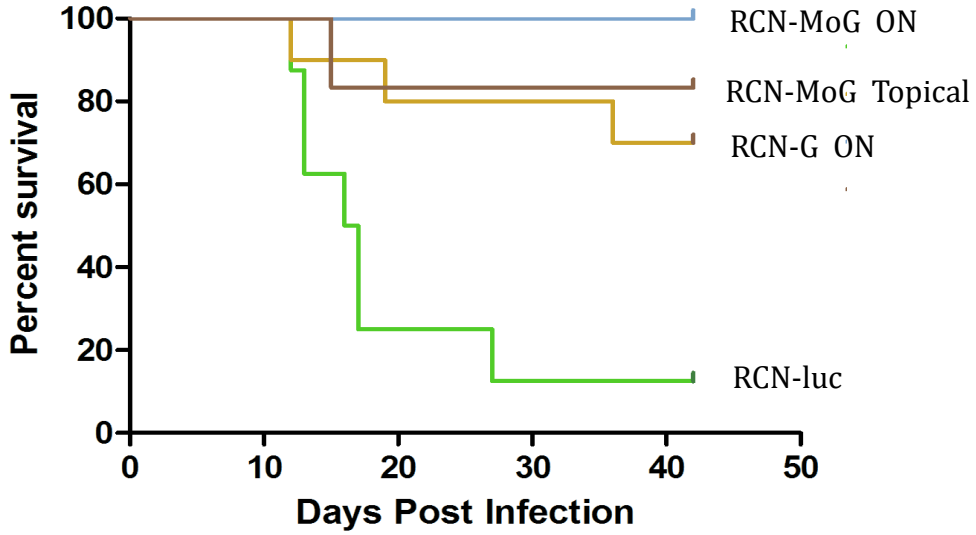

Figure 3. Results of vaccine efficacy and rabies challenge trials in *Epstesicus fuscus* immunized with raccoon poxvirus expressing a mosaic G protein (RCN-MoG) either oronasally (ON) or topically in comparison to RCN expressing typical G protein and RCN expressing luciferase (a negative control).

For bats a different approach is required for vaccine delivery, as in general, they are not attracted to baits. Bats, especially vampire bats, are known to practice self and mutual grooming at a high rate, and this behavior has been exploited to cull vampire bats using poisons like warfarin. The poison is applied topically to a number of bats that are released. When they return to their roost, the poison is transferred to roost-mates by contact and mutual grooming. We are exploiting this same behavior for vaccine application. Preliminary biomarker studies (without vaccine) are being conducted in vampire bats in both Mexico and Peru and also in insectivorous bats in Wisconsin. In a pilot study in Peru, we treated 50 bats from a single cave with RB-labelled glycerin jelly. Based on capture-recapture data, we estimated the population at ~200 bats, so ~25% of bats were initially marked. Upon trapping of this population a few days later, 64 bats were captured, including 19 originally marked bats (Table 1 – could be made into a figure instead). Hair was collected and examined for RB marking under a fluorescence microscope. All treated bats were positive for RB marking in addition to 39% of newly captured bats, indicating a rate of transfer of about 1.3 bats for every bat marked. Additional trials have been conducted, with transfer rates of up to 2.8 bats for every bat treated achieved at least once. These trials are being analyzed to assess factors associated with rates of transfer, e.g. sex and age of initially treated bats, time of day, etc. This data is then being used to model the rate of vaccination and impact on rabies transmission with different rates of application, prior to actual deployment of vaccine in the field.

Table 1. Marking of vampire bats a few days after application of glycerin jelly containing Rhodamine B.

	Number captured	Positive	Negative	Inconclusive	% positive (w/o inc)
All bats	64	34	25	5	58
Recaptured marked bats	19	18	0	1	100
New bat captures	45	16	25	4	39

For insectivorous bats, we are trying other approaches. Instead of hand applying the jelly to bats, we applied RB marked glycerin jelly to the entry of bat houses used by little brown bats (*Myotis lucifugus*). The bats became covered as they entered the houses and then consumed the material during self and mutual grooming. One week later, bats were trapped at the houses to determine the rate of uptake. Of 29 bats trapped one week post-application, 59% (17) were positive for biomarker indicating they had eaten the jelly. Thus, with additional optimization, application of vaccine to bat houses or other structures (small cave entrances) could also be a viable method of delivery. In addition, we are considering different spray applications directly to roosting bats in caves and through motion-sensing sprayers at cave entrances. Whatever the means of application, effective treatment relies on ingestion by bats, and that is easily confirmed with the use of the biomarker, RB.

PARC will develop the FEA aerosol technology wide-scale inoculation of bats in PRE-EMPT. Fig.4 shows the basic principle of the technology and the resulting spray from representative fluids (aqueous polymer solutions, consumer formulations). FEA technology can be used for the full range of fluids of interest to the program including gels and creams for topical application and aqueous/non-aqueous vaccine formulations. Further details can be found in the PARC website (see references).

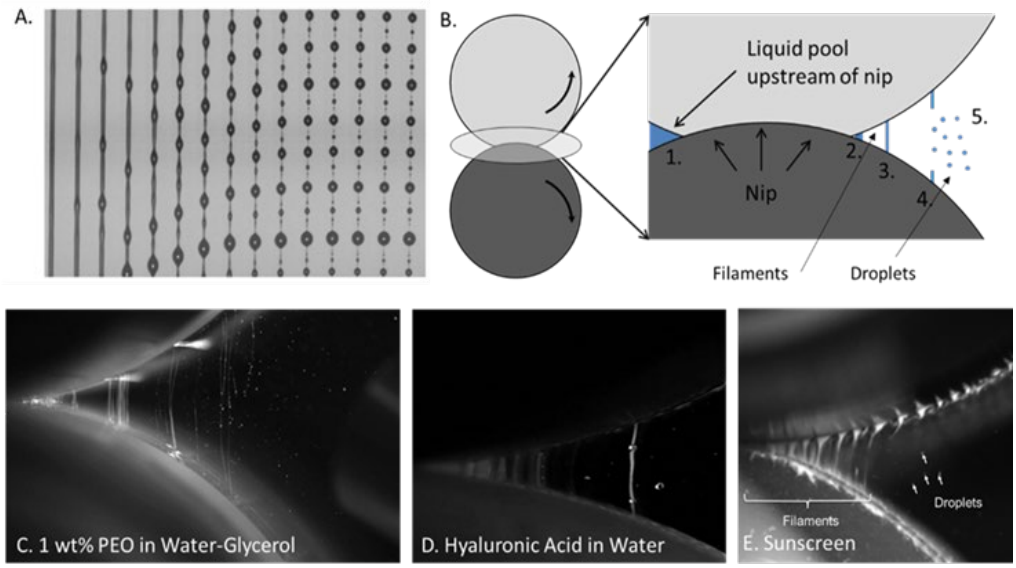

Figure 4. FEA technology: A. Beads-on-a-string formation in viscoelastic fluids in extension (Oliveira and McKinley, 2005), B. Roll-to-roll parallelization of filament formation and break-up in FEA, C.-E. Examples of fluids sprayed with FEA including polyethylene oxide in water-glycerol (C.), hyaluronic acid in water (D.) and sunscreen (E.)

Organization leading task: USGS National Wildlife Health Center
Participating organizations: Palo Alto Research Center (PARC)

Progress Metrics: Not sure exactly what format to use here

Deliverable(s):
Medium and methods to deliver immunomodulatory agents to bats.
Data on uptake in insectivorous bats.
Reports, manuscripts, presentations.

Coleman CM, Liu YV, Mu H, Taylor JK, Massare M, Flyer DC, Smith GE, Frieman MB. 2014. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine 32:3169-3174.

Ebrahimian M, Hashemi M, Maleki M, Hashemitabar G, Abnous K, Ramezani M, Haghparast A. 2017. Co-delivery of dual toll-like receptor agaonists and antigen in poly(lactic-co-glycolic) acid/polyethylenimine cationic hybrid nanoparticles promote efficient in vivo immune responses. Front Immunol 8:1077.

Freuling CM, Hampson K, Selhorst T, Schro¨der R, Meslin FX, Mettenleiter TC, Mu¨ller T (2013) The elimination of fox rabies from Europe: determinants of success and lessons for the future. Philosophical Transactions of the Royal Society London B Biological Sciences 368(1623):20120142 (DOI: 10.1098/rstb.2012. 0142)

Karande P, Mitragotri S. 2010. Transcutaneous immunization: an overview of advantages, disease targets, vaccines, and delivery technologies. Annu Rev Chem Biomol Eng 1:175-201.

Mishra DK, Dhote V, Mishra PK. 2013. Transdermal immunization: biological framework and translational perspectives. Expert Opin Drug Deliv 10:183-200.

Oliveira MSN, McKinley GH. 2005. Iterated stretching and multiple beads-on-a-string phenomena in dilute solutions of highly extensible flexible polymers. Physics of Fluids 17: 071704.

PARC Website: Advanced Manufacturing and Deposition Systems Group – https://www.parc.com/services/focus-area/amds/

Roberts MS, Mohammed Y, Pastore MN, Namjoshi S, Yousef S, Alinaghi A, Haridass IN, Abd E, Leite-Silva VR, Benson HAE, Grice JE. 2017. Topical and cutaneous delivery using nanosystems. J Control Release 247:86-105.

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Slate D, Algeo TP, Nelson KM, Chipman RB, Donovan D, Blanton JD, Niezgoda M, Rupprecht CE (2009) Oral rabies vaccination in North America: opportunities, complexities, and challenges. PLoS Neglected Tropical Diseases 22 3(12):e549.doi:10.1371/journal.pntd.0000549

Stading BR, Osorio JE, Velasco-Villa A, Smotherman M, Kingstad-Bakke B, Rocke TE. Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). Vaccine. 2016;34: 5352–5358. doi:10.1016/j.vaccine.2016.08.088

Stading B, Ellison JA, Carson WC, Panayampalli SS, Rocke TE, Osorio JE. Protection of bats (*Eptesicus fuscus*) against rabies following topical or oronasal exporue to a recombinant raccoon poxvirus vaccine. PLoS Negl Trop Dis 11:e0005958.

Tripp DW, Rocke TE, Streich SP, Brown NL, Fernandez JR-R, Miller MW. 2014. Season and application rates affect vaccine bait consumption by prairie dogs in Colorado and Utah, USA. J Wildlife Dis 20:

Tripp DW, Rocke TE, Streich SP, Abbott RC, Osorio JE, Miller MW. 2015. Apparent field safety of a raccoon poxvirus-vectored plague vaccine in free-ranging prairie dogs, Colorado, USA. J Wildlife Dis 51:

DARPA DEFUSE draft v2 - Technical Comment

Peter Daszak <daszak@ecohealthalliance.org>

Thu 3/22/2018 12:53 AM

To: Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; Rocke, Tonie E <trocke@usgs.gov>; Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>
Cc: Danielle Anderson (danielle.anderson@duke-nus.edu.sg) <danielle.anderson@duke-nus.edu.sg>; aaron.irving@duke-nus.edu.sg <aaron.irving@duke-nus.edu.sg>; antonette_baric@med.unc.edu <antonette_baric@med.unc.edu>; sims0018@email.unc.edu <sims0018@email.unc.edu>; Luke Hamel <hamel@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>

Dear all,

Apologies for the delay - here's the draft Technical Plan for our proposal with everyone's section incorporated, edited and shortened.

Please ignore all other sections – these are being worked on by others. We're only editing the Technical Plan right now.

Can each of you go through your respective section and, with one of you acting as the point person to coordinate edits and responses from your teams:

1. Answer any questions in comment boxes

2. Insert any missing references – please just cut and paste the ref as a word doc into a comment box rather than inserting the endnote reference at this point

3. Read through your sections and suggest edits. Best if you use lots of comment boxes, but also OK if you start editing using 'track changes'. NB – we need to reduce the length probably by one third, so any suggestions and cuts would be most appreciated! Also, please just keep this as a Word doc for now – there are formatting issues when converting backwards and forwards into Google docs and Word.

4. Ralph and team – please provide higher res images for all those in this draft, and please make sure they're editable – i.e. we can take out the text and alter each icon within each image

5. All – please check the language I've used and correct any glaring errors.

6. Can you get edits back to me, cc'd to Luke and Anna by **Saturday 9am Eastern (New York) time,** at which point I'll start trimming it back into the page limit and incorporating all the other sections.

While you're working on these sections, I'll be editing the rest of the proposal with Luke, Anna and others.

Cheers,

Peter

Peter Daszak
President

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@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

-----Original Message-----
From: Peter Daszak
Sent: Tuesday, February 27, 2018 2:14 PM
To: Zhengli Shi (zlshi@wh.iov.cn); Ralph Baric (rbaric@email.unc.edu); 周鹏 (peng.zhou@wh.iov.cn); 'Wang Linfa'; Rocke, Tonie
Cc: Danielle Anderson (danielle.anderson@duke-nus.edu.sg); 'aaron.irving@duke-nus.edu.sg'; 'antonette_baric@med.unc.edu'; 'sims0018@email.unc.edu'; Luke Hamel (hamel@ecohealthalliance.org)
Subject: For our DARPA PREEMPT conversations this week: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status
Importance: High

Dear All,

Good news from DARPA - they like our abstract and we're officially invited for a full proposal. From the attached letter, it looks like they've got a lot of proposals asking for too much $$$, but there are some clear ways we can hedge against any possible cuts. We can talk further about this, and about fleshing out the technical details on our calls this week.

I'm working on scheduling a call with the DARPA team for Thursday of Friday this week - 15 mins to go through how these bullets in the letter above will affect our full proposal. It'll just be me and Luke, but we can think about key questions to ask them..

Re. the full proposal. Luke has taken the abstract text and started populating the full proposal framework (attached), to give us an idea of what we need to write. It's not a huge effort, but it'll have to be technically sound, but still tell the overall 'story' that DARPA want to hear - i.e. we can provide proof-of-concept of blocking spillover based on this novel and interesting approach.

Look forward to talking with all of you.

Cheers,

Peter

Peter Daszak
President

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

-----Original Message-----
From: PREEMPT [mailto:PREEMPT@darpa.mil]
Sent: Tuesday, February 27, 2018 8:51 AM
To: (b) (6)
Cc: (b) (6))
Subject: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status

(b) (6)

Thank you for your interest in the Biological Technologies Office's PREventing EMerging Pathogenic Threats (PREEMPT) program. Please find your proposal abstract status attached.

Regards,

BAA Coordinator
Contractor Support to DARPA/BTO
PREEMPT@darpa.mil

A. EXECUTIVE SUMMARY

Technical Approach: Our goal is to defuse the potential for spillover of novel bat-origin high-zoonotic risk SARS-related coronaviruses in Southeast Asia. **In TA1** we will develop **host-pathogen ecological niche models** to predict the species composition of bat caves across Southeast Asia. We will parameterize this with a full inventory of host and virus distribution at our field sites, three caves in Yunnan Province, China and a series of unique datasets on bat host-viral relationships. By the end of Y1, we will use these to create a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens at any site across Asia. We will intensively sample bats at our field sites to sequence SARSr-CoV spike proteins, reverse engineer them to conduct binding assays, and insert them into SARS-CoV backbones to infect humanized mice to assess capacity to cause SARS-like disease. Our modeling team will use these data to build **machine-learning genotype-phenotype models** of viral evolution and spillover risk. We will uniquely validate these with human serology data through LIPS assays designed to assess which spike proteins allow spillover into people.

In TA2, we will evaluate two approaches to reduce SARSr-CoV shedding in cave bats: (**1) Broadscale Immune Boosting**, in which we will inoculate bats with immune modulators to upregulate their innate immune response and downregulate viral replication; **(2) Targeted Immune Priming**, in which we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance innate immunity against specific, high-risk viruses. We will trial inoculum delivery methods on captive bats including automated aerosolization, transdermal nanoparticle application and edible, adhesive gels. We will use stochastic simulation modeling informed by field and experimental data to characterize viral dynamics in our cave sites, to maximize timing, inoculation protocol, delivery method and efficacy of viral suppression. The most effective delivery method and treatments will be trialed in our experimental cave sites in Yunnan Province, with reduction in viral shedding as proof-of-concept.

Management Approach: Members of our collaborative group have worked together on bats and their viruses for over 15 years. The lead organization, EcoHealth Alliance, will oversee all modeling, lab, and fieldwork. EHA staff will develop models to evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for delivery of both immune boosting and immune targeting inocula. Specific work will be subcontracted to the following organizations:

- Prof. Ralph Baric, UNC, will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades.
- Prof. Linfa Wang, Duke-NUS, will lead work on immune boosting, building from his groups' pioneering work on bat immunity.

- Dr. Zhengli Shi, Wuhan Institute of Virology will conduct viral testing on all collected samples, binding assays and some humanized mouse work.
- Dr. Tonie Rocke, USGS National Wildlife Health Center will develop a delivery method for immunological countermeasures, following from her work on vaccine delivery in wildlife, including bats.
- XXX, PARC – Leading development of novel delivery mechanism

B. EXECUTIVE SUMMARY SLIDE

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C. GOALS AND IMPACT

Overview

The overarching goals of DEFUSE are:

- **Identify and model the spillover risk** of novel SARS-related CoVs in South and SE Asia
- Design and demonstrate proof-of-concept that interventions to upregulate the naturally low innate immunity of bats to viruses (**immune boosting**) and to high risk SARSr-CoVs in particular (**immune priming**) will transiently reduce spillover risk.

We will analyze, design and field-test a novel strategy to reduce risk of viral emergence from bats that will help protect the warfighter within SACOM and SEACOM, and will be scalable to other systems including Ebola virus, rabies and other bat-origin pathogens.

Commented [PD1]: Check on correct DoD names for these regions

Innovation and uniqueness:

Bats harbor more emerging zoonoses than any other group of mammals, and are ubiquitous,

abundant, wide-ranging and often overlooked. Despite this, other than PPE, there is no available current technology to reduce the risk of exposure to novel coronaviruses from bats. Models of bats' capacity to harbor viruses, of ecological and environmental drivers of their emergence, and of the evolutionary potential of different strains to spillover are rudimentary. No vaccines or therapeutics exist for SARSr-CoVs, and exposure mitigation strategies are non-existent. SARSr-CoVs are enzootic in Asian, African[1], and European bats[2] that roost in caves but forage widely at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have recently shown evidence of spillover of SARSr-CoVs into people in China, unrelated to the original SARS pandemic, and have isolated strains capable of producing SARS-like disease in humanized mice that don't respond to antibody treatment or vaccination. These viruses are a clear-and-present danger to our military and to global health security because of their continuous circulation and evolution in bats and periodic spillover into humans in locations where surveillance is virtually nonexistent.

Commented [PD2]: There's a new ref that I tweeted about recently - http://coronavirus.fr/publications/

EcoHealth Alliance leads the world in predictive models of viral emergence. We will build on our machine-learning models of spillover hotspots, host-pathogen ecological niche and genotype-phenotype mapping by incorporating unique datasets to validate and refine hotspot risk maps of viral emergence in SE Asia and beyond. We have shown that bats are able to carry otherwise lethal viruses by virtue of dampened innate immunity (e.g. inflammatory) pathways, which likely evolved as an adaptation to the physiologic stress of flight. We will use this insight to design strategies, like small molecule Rig-like receptor (RLR) or Toll-like receptor (TLR) agonists, to upregulate bat immunity and down-regulate viral replication in their cave roosts, thereby significantly reducing the frequency and magnitude of viral shedding and spillover **(broadscale immune boosting strategy)**. We will complement this by treating bats with novel chimeric polyvalent recombinant spike proteins to enhance their adaptive immune response against specific, high-risk coronaviruses **(targeted immune priming strategy)**, especially when their innate immune response is boosted as above. We will design novel automated application methods, based on our previous work delivering wildlife vaccines, to apply these interventions in a way that eliminates the need for a person to enter a cave and potentially get exposed to bat borne viruses or other hazards.

Technical Area 1

Our strategy to reduce spillover risk of bat SARS-related CoVs begins with modeling to predictively assess spillover risk across South and SE Asia using baseline genotype-phenotype analysis of host and strain diversity from the literature, from surveillance in our designated model caves in China, and across the region in other projects. In TA1, the DEFUSE modeling and analytics team, will build joint species distribution models (JSDM) of environmental and ecological correlates and traits of cave bat communities to predict species composition of bat caves across Southern China, South and SE Asia. Dr. Epstein at EHA will coordinate animal

experimental work with the teams at NWHC, Duke-NUS and Wuhan and radio telemetry studies with the field surveillance team. We will then use a series of datasets we have built to produce host-virus risk models for the region. These include our comprehensive database of bat host-viral relationships and estimates of zoonotic viral richness per bat species[3]; biological inventory data on all bat caves in Southern China; and modeled species distribution data for all bats. We will parameterize the model with data from three cave sites in Yunnan, China (one with high-risk SARSr-CoVs, two other control/comparison sites), including: radio- and GPS-telemetry to identify home range and additional roost sites for each bat species; inventory of bat population density, distribution and segregation and their daily, weekly and seasonal changes; viral prevalence and individual viral load; shedding of low- and high-risk SARSr-CoV strains among bat species, age classes, genders; and telemetry and mark-recapture data to assess metapopulation structure and inter-cave connectivity. We will test and validate model predictions of a cave's viral spillover potential with data from prior PREDICT sampling in 7 other Asian countries. At the end of Yr 1, we will produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens in a region. **The 'Spatial viral spillover risk' app** will be updated real-time with surveillance data (e.g. field-deployable iPhone and android compatible echolocation data) from our project and others, to ground-truth and fine-tune its predictive capacity.

The Wuhan Institute of Virology team will test bat fecal, oral, and blood samples for SARSr-CoVs. We will collect viral load data using fresh fecal pellets from individually sampled bats and from tarps laid on cave floors deployed where necessary to reduce roost disturbance. SARSr-CoV spike proteins will be sequenced, analyzed phylogenetically for recombination events, and high-risk viruses (spike proteins close to SARS-CoV) characterized and isolated. The UNC team will reverse-engineer spike proteins to conduct binding assay to human ACE2 (the SARS-CoV receptor). They will culture SARS-like bat coronaviruses to distinguish high-risk strains that can replicate in primary human cells and low risk strains that require exogenous enhancers. Viral spike glycoproteins that bind receptors will be inserted into SARS-CoV backbones, inoculated into human cells and humanized mice to assess capacity to cause SARS-like disease, and to be blocked by monoclonal therapies, the nucleoside analogue inhibitor GS-5734[4] or vaccines against SARS-CoV[4-8].

The EHA modeling team will use these data to **build models of risk of viral evolution and spillover**. These genotype-to-phenotype machine-learning models will predict viral ability to infect human host cells based on genetic traits and results of receptor binding and mouse infection assays. Using data on diversity of spike proteins, recombinant CoVs, and flow of genes within each bat cave via bat movement and migration, we will estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Finally, virus-host relationship and bat home range data will be used to estimate spillover potential - extending models well beyond our field sites. We will then **validate model**

Commented [3]: Are we saying only one cave site has SARSr-CoVs, or does one site have a higher prevalence of these compared to controls? Should validate this with our prelim data if possible. Are 3 sites sufficient?

Commented [4]: no need for urogenital samples and these bats are too small to collect those anyway. Fecal and oral are key. Blood is also important for serolgy

Commented [5]: Edited this slightly, but we could just go with individually sampled bats, should be easy enough to say we'll sample ~200 individually trapped bats on a monthly basis at cave entrances using harp trap, if we want to do away with tarp sampling. Alternatively we could leave both and justify the use of tarps and that we'll use high-resolution photos of bats roosting in caves to estimate population size from populations sampled non-invasively using tarp collection.

predictions of viral spillover risk by **1)** conducting spike protein-based binding and cell culture experiments, and **2)** identifying spillover strains in people near our bat cave sites. Our preliminary work on this shows ~3% seroprevalence to SARSr-CoVs, using a specific ELISA [REF]. We will design LIPS assays to the specific high- and low- zoonotic-risk SARSr-CoVs identified in this project as we have done previously [REF]. We will use previously collected and newly collected human sera from these populations to test for presence of antibodies to the high- and low-risk SARSr-CoVs identified by our modeling. We will then **model optimal strategies to maximize treatment efficacy** for TA2, using stochastic simulation modeling informed by field and experimental data to characterize viral circulation dynamics in bats. We will estimate frequency and population coverage required for our intervention approaches to suppress viral spillover. We will determine the seasons, locations within a cave, and delivery methods (spray, swab, or automated cave mouth or drone) that will be most effective. Finally we will determine the time period treatment will be effective for, until re-colonization or evolution leads to return of a high-risk SARSr-CoV.

Technical Area 2

In TA2, we will **develop scalable approaches that target and suppress the animal virus in its reservoir(s)and/or vector(s), to reduce the likelihood of virus transmission into humans**. We will evaluate two approaches to defuse SARS-related CoV spillover potential**: 1) Broadscale Immune Boosting:** using the unique immune damping in bats that our group has discovered, we will apply immune modulators like bat interferon to live bats, to up-regulate their naïve immunity and then assess their ability to suppress viral replication and shedding; **2) Targeted Immune Priming:** building on preliminary development of polyvalent chimeric recombinant SARSr-CoV spike proteins, we will conduct application trials with live bats to assess suppression of replication and shedding of a broad range of dangerous SARS-related CoVs.

Both lines of work will begin in Yr 1 and run parallel. Prof. Linfa Wang (Duke-NUS) will lead the immune boosting work, building on his pioneering work on bat immunity[9] which shows that the long-term coexistence of bats and their viruses has led to equilibrium between viral replication and host immunity. This is likely due to down-regulation of their innate immune system as a fitness cost of flight[9]. The weakened functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may allow bats to maintain an effective, but not over-response to viruses[10]. A similar finding was observed for bat IFNA, which is less abundant but constitutively expressed without stimulation[11]. Given high native SARSr-CoV load in bats, we aim to boost bat innate immunity through the IFN pathway, break the host-virus equilibrium to suppress bat SARSr-CoV replication and shedding.

We will trial the following, concurrently and competitively, for efficiency, cost and scalability: **i)** Universal bat interferon. Aerosol spraying or intranasal application of IFN or other small molecules reduces viral loads in humans, ferrets and mouse models[12,13]. Interferon has

been used clinically when antiviral drugs are unavailable, e.g. against filoviruses[14]. Replication of SARSr-CoV is sensitive to interferon treatments, as shown in our previous work[13]; **ii)** Boosting bat IFN by blocking bat-specific IFN negative regulators. Uniquely, bat IFNA is naturally constitutively expressed but cannot be induced to a high level[11], indicating a negative regulatory factor in the bat interferon production pathway. We will use CRISPRi to identify the negative regulator and then screen for compounds targeting this gene; **iii)** Activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7-dependent pathways. Our work showing that mutant bat STING restores antiviral functionality suggests these pathways are important in bat-viral coexistence[10]. By identifying small molecules to directly activate downstream of STING, we will activate bat interferon and promote viral clearance. A similar strategy will be applied to ssRNA-TLR7-dependent pathways; **iv)** Activating functional bat IFN production pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been demonstrated in a mouse model for SARS-CoV, IAV and HBV[12,15]; **v)** Inoculating crude coronavirus fragments to upregulate innate immune responses to specific CoVs – a partial step towards the targeted immune priming work below.

Prof. Ralph Baric (UNC) will lead the immune priming work. He will develop recombinant chimeric spike-proteins[16]from our known SARSr-CoVs, and those we characterize during project DEFUSE. The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain[17]. In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles[6,18-21]. We will test these in controlled lab conditions, taking the best candidate forward for testing in the field. We have built recombinant spike glycoproteins harboring structurally defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broad scale immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, potentially reducing disease risk (and transmission risk to people) in these animals for longer periods[22,23].

The immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established the only experimental breeding colony of cave bats (*Eonycteris spelaea*) in SE Asia. This genus is evolutionarily related to *Rhinolophus* spp. (the hosts of SARSr-CoVs), so we have confidence that results will be transferable. Our initial proof-of-concept tests will be in this experimental colony, extended to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with *Rhinolophus* sp. bats in the BSL-4 facility at CSIRO, AAHL (L.Wang, unpublished results).

Commented [AW6]: Is this our work? ref may be wrong

Finally, work on a delivery method for our immune boosting and priming molecules will be developed and implemented by Dr. Tonie Rocke at the USGS, National Wildlife Health Center who has previously developed animal vaccines through to licensure[24]. Using locally acquired insectivorous bats[25,26], we will assess delivery vehicles and methods including: 1) transdermally applied nanoparticles; 2) series of sticky edible gels that bats will groom from themselves and each other; 3) aerosolization via sprayers that could be used in cave settings; 4) automated sprays triggered by timers and movement detectors at critical cave entry points, and 5) sprays delivered by remote controlled drone. We have already used simple gels to vaccinate bats against rabies in the lab[25], and hand delivered these containing biomarkers to vampire bats in Peru and Mexico to show they are readily consumed and transferred among bats. In our bat colony, we will trial delivery vehicles using the biomarker rhodamine B (which marks hair and whiskers upon consumption) to assess uptake. The most optimal approaches will then be tested on wild bats in our three cave sites in Yunnan Province with the most successful immunomodulators from TA2. Fieldwork will be conducted under the auspices of Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance). A small number of bats will be captured and assayed for viral load and immune function after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has had unique access to these sites for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for experimental trials from the Provincial Forestry Department. We expect to be successful, as we have worked with the Forestry Department collaboratively for 10 years, with support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife. EHA has a proven track record of rapidly obtaining IACUC and DoD ACURO approval for bat research.

Deliverables:

- App identifying geographical risk of spillover for novel SARSr-CoVs in SE Asia
- Identified indicators (modeled and validated) of spillover capacity for different viral strains.
- Proven mechanistic approach to modulating bat innate immunity to reduce viral shedding
- Tested and validated delivery mechanism for bat cave usage including vaccines in other bat host-pathogen systems (e.g. rabies, WNS).
- Proof-of-concept approach to transiently reducing viral shedding in wild bats that can be adapted for other systems including Ebola virus.

D. TECHNICAL PLAN

Technical Area I:

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Choice of site and model host-virus system. For the past 14 years, our team has conducted coronavirus surveillance in bat populations across Southern China, resulting in <150 CoV identifications in ~10,000 samples[27-29]. Bat SARSr-CoVs are genetically diverse, especially in the S gene, and most are highly divergent from SARS-CoV. However, in a cave site complex in Yunnan Province, we have found bat SARSr-CoVs with S genes extremely similar to SARS-CoV, and which, as a quasispecies population assemblage contain all the genetic components of epidemic SARS-CoV[30].

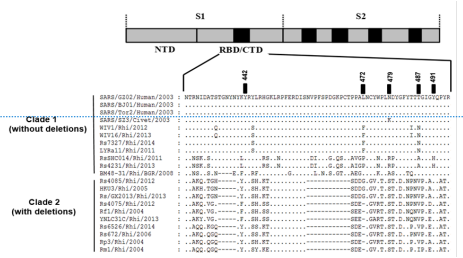

Fig. 1: Alignment of amino acid sequence of the receptor-binding motif in the spike protein of SARSr-CoVs and SARS-CoV[30]. Numbered amino acid is the key residues which is responsible for SARS-CoV S and human ACE2 interaction[31].

We have isolated three strains at this site (WIV1, WIV16 and SHC014) that unlike other SARSr-CoVs, do not contain two deletions in the receptor-binding domain (RBD) of the spike, and share substantially higher sequence identity to SARS-CoV (Fig. 1). These viruses have been demonstrated to use human ACE-2 receptor for cell entry as SARS-CoV does (Fig. 2), and replicate efficiently in various animal and human cells[27,29,30,32,33] including primary human lung airway cells, similar to epidemic SARS-CoV[7,8].

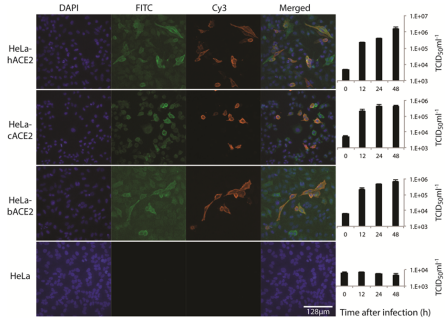

Fig. 2: Bat SARSr-CoV WIV1 replicates efficiently in HeLa cells expressing human, civet and bat ACE2[29].

Chimeras (recombinants) with these SARSr-CoV S genes inserted into a SARS-CoV backbone, as well as synthetically reconstructed full length SHCO14 and WIV-1 bat viruses cause SARS-like illness in humanized mice (a model that expresses human ACE2 receptor), with clinical signs that are not reduced by SARS-CoV monoclonal antibody therapy or vaccination[7,8]. We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort)[34], suggesting active spillover. These data, phylogeographic analysis of SARSr-CoVs (Fig. 3), and coevoutionary analysis of bats and their CoVs (unpubl. data), suggest that bat caves in SW China, and *Rhinolophus* spp. bats are the likely origin of the SARS-CoV clade, and **therefore a**

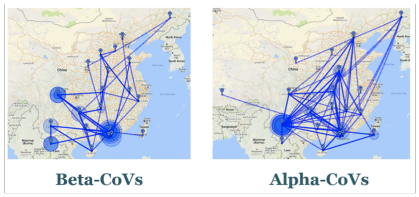

Figure 3. Ancestral location reconstruction for Beta- and Alpha-CoVs. The bigger the circle is, the more ancestral the corresponding node is.

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clear-and-present danger for the re-emergence of SARS-CoV or a similar pathogenic virus. The *Rhinolophus* spp. bats that harbor these viruses occur throughout SE Asia, across S. and W. Asia. **Thus, the geographic focus of DEFUSE is to use our research at this site to reduce the risk for the warfighter of these viruses spilling over across the region (West, South and SE Asia).**

Spatial models of bat origin high-risk viruses across S and SE Asia. We will build models that predict regional-scale bat and viral diversity in cave sites across South and SE Asia to enable warfighters and planners to estimate regional-scale risk from viral spillover based on locations. This will provide preliminary assessments for areas requiring greater on-the ground risk characterization to target deployment of viral suppression technologies. These regional-scale joint species distribution models (JSDM) will predict the composition of bat communities in caves in South Southern China, South and SE Asia. JSDMs use environmental and habitat data to predict the distributions of many species simultaneously, producing more accurate predictions than individual, separate species predictions by explicitly modeling positive and negative interactions between species and hidden factors such as shared habitat preferences. We will use a stochastic feedforward neural network to implement JSDMs that has proven effective at making predictions across multiple scales, with incomplete observations (as occurs for bats and their viruses), and explicitly accounting for bat species co-occurrence driven by shared environmental responses or evolutionary processes[35]. We will fit our JSDM to biological inventory data on over 200 caves in the region[36], using a combination of climatic and topographic variables including physiologically relevant bioclimatic variables (BIOCLIM) drawn from public, open source data sets[37], as well as proxies for subterranean habitat such as ruggedness and habitat heterogeneity. We will refine these models using regional-scale environmental variables (land-use, distance to roads, forest cover, degree of human disturbance etc.) and cave-specific variables (cave length, availability of roosting area, entrance dimensions, cave complexity, microclimate etc.). Our previous work has shown that these factors are predictors of bat species presence/absence at a given site[38]. Remote-sensing data and physical models will be used to estimate cave structures and microclimates where they are not available from biological inventory studies. We will validate our regional-scale species models using independent occurrence estimates and observations[39,40], including our extensive database on bat species occurrence in Southeast Asia [REF].

We will extend our predictions of bat communities to predictions of zoonotic disease risk using our unique species-level database of all known bat host-viral relationships[3] (Fig. 4); our >1800 viral detections from >20,000 individual bat samples in China and 7 other Asian countries (NIAID and USAID PREDICT); and results as they become available from a new 5-year DTRA-CBEP grant for field and lab investigations to characterize bat CoV diversity in Western Asia (Turkey, Jordan, Georgia, Pakistan, and Arabian Peninsula – EHA, Olival) to extend the geographic scope of our predictive models. We will use two strategies to predict presence of

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viruses at sites. Firstly, as a base case, we will assume that species have equal probability of carrying their known viral species across their range. Second, we will include viral species as additional outputs in our JSDM. We will fit this host-viral JSDM using data restricted to a smaller set of sites where both host species composition and viral detections are available. Based on performance of both models on hold-out data, we will determine which provides the best predictive power. For species composition and viral presence predictions, we will validate our models against a 20% validation subset of data that is held out for model validation, as well as data collected at our field sites in Task 3.

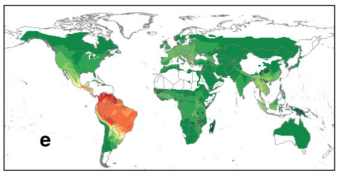

*Fig. 4: Predictive global map of total (known and unknown) viral diversity in bats (*Chiroptera *species). Based on EHA's unique database of all known mammal virus-host relationships[3].*

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Prototype app for the warfighter. Drawing on experience building applications for data collection and analysis (e.g. https://flirt.eha.io/, https://eidr-connect.eha.io/, https://mantle.io/grrs), we will produce a prototype app for the warfighter that identifies the likelihood of dangerous viral pathogens spilling over from bats at a site. **The 'Viral spillover risk' app** will use outputs from our spatial risk modeling, data from EHA's extensive host-pathogen database, open-source species and pathogen ontologies, and app-directed crowd-sourced ultrasonic audio recordings to ground-truth and fine-tune its predictive capacity. This app will be updated in Y2 and Y3 to incorporate additional information on bat species-specific risk based on assays of host-virus binding and surveys of CoV prevalence. We will use risk-ranking algorithms developed by EHA (https://ibis.eha.io/) that use geolocation features, recency of information, and host and pathogen characteristics to display critical areas of high risk. The app will collect user GPS location data and preload bat species distribution and community composition estimates from our JSDMs. These will be refined with real-time surveillance data collected without the need to enter cave sites using field-deployable high-frequency microphones for bat detection[41]. We will combine reference acoustic calls from all bat species captured during proposed field work with existing data from bat call libraries globally to train species identification algorithms using bat echolocation call signatures. New algorithms using deep learning methods (e.g. convolutional neural networks[42]) will be developed, or adapted and externally validated on samples collected by the application to characterize bat species based on trained audio features. These models will be deployed on the mobile platform as they become available[42]. Bat species directly identified or estimated to occur within a scalable distance from the user will be automatically linked with viral diversity data from EHA's extensive host-pathogen database and with CoV sequence data from this project to deliver high-risk pathogen lists. The application will have 3 primary views; pathogens-centric, bat-centric and map-centric. The pathogen-centric view will show a ranked list of likely

pathogens in the user's current or selected location. The bat-centric view will show a ranked list of bat species for the user's location. The map-centric view will allow users to select a location for the other rank views, and will display a variety of map layers of interest, including heat map or distribution map layers profiling modeled or collected species occurrences around the user. Elements of the interface will be interactive, presenting popovers with more details when selected and displaying other map elements as appropriate. Alerts and notifications will give users a flexible way to monitor the app data passively, with the app proactively reaching out when critical information is received. The application will also offer a data collection module and accompanying interface elements to collect samples in the field and integrate collected data into the application database. The schemas, APIs, and protocols developed as part of this effort will be designed with principles of simplicity, interoperability, and usability in mind, including using RESTful URL schemes, and standardized data types and ontologies. Datasets will be hosted via cloud services from which the app will download updated information. Build and deployment processes will be reproducible, auditable, and transparent. All code modules will be continually available on EHA's GitHub page (LINK), be documented via README files in root directory of code repositories, and .zip archives containing code, datasets, and instructions for deployment will be made available. This will pave the way future incorporation of new structured biosurveillance data feeds and new species, viral, or host ontologies. This app will be designed for remote use (desktop platform) to assess specific sites in advance of personnel deployment on the ground, or in the field via mobile systems. This technology will improve overall situational awareness of existing and novel infectious agents found in bats, allowing DoD personnel to quickly identify areas that may pose the most significant risk for zoonotic spillover and rapidly deploy resources to respond to and mitigate their impact preemptively when necessary. The 'viral spillover risk' app will then be available to adapt for viral threats from other wildlife host species (e.g. rodents, primates) and ultimately for global use.

Full inventory of bat SARSr-CoV quasispecies at our cave test sites, Yunnan, China.
DEFUSE fieldwork will focus on three model cave test sites within a cave complex in Yunnan Province, SW China (MAP), where we have previously identified and isolated high-risk SARSr-CoVs able to infect human cells and cause SARS-like illness in mice[7,27,29,30]. At these sites, we will determine the baseline risk of SARSr-CoV spillover, prior to, during, and after our proof-of-concept field trials to reduce that risk. We will conduct longitudinal surveillance of bat populations to detect and isolate SARSr-CoVs, determine changes in viral prevalence over time, measure bat population demographics and movement patterns, to definitively characterize their SARSr-CoV host-viral dynamics. We will sample *Rhinolophus, Hipposideros*, and *Myotis* species, all of which carry SARSr-CoVs, and co-roost in the same caves[3,36]. Surveillance will be conducted before, during, and after deployment of our intervention field trial (Task X) to establish baseline viral shedding detection rates and measure the impact of treatment on

these. Field data will allow us to test the accuracy of our model predictions and compare the efficacy of laboratory trials in animal models with in-the-field trials.

Our test caves near Kunming, Yunnan Province, contain multiple co-roosting *Rhinolophus*, *Hipposideros*, and *Myotis* spp., although our preliminary data demonstrate that *R. sinicus* and *R. ferrumequinum* (which co-roost at our sites) are the SARSr-CoV primary reservoir, with *Hipposideros* and *Myotis* playing an insignificant role in viral dynamics. We will capture bats using harp traps and mist nets during evening flyout. Rectal, oral, and whole blood samples (×**2** per bat) will be collected for viral discovery using sterile technique to avoid cross-contamination. 2-mm wing tissue punch biopsies will be collected from each bat for host DNA bar-coding, sequencing of host ACE-2 receptor genes (interface site), and cophylogeny analyses. Standard morphological and physiological data will be collected for each bat (age class, sex, body weight, reproductive status etc.). In Phase I we will **sample 60** *Rhinolophus sinicus* and **60** *R. ferrumequinum*, our primary target species, (**120** bats total) **every three months** for non-lethal viral specimen collection over an **18 month period** of the project from all three cave sites. Given the average prevalence of SARSr-CoV in these species in our previous investigations in S. China (~6-9%, n=3304 *Rhinolophus* spp.), this sample size would enable to detect changes of 10% fluctuation in prevalence between sampling periods. Early in the sampling we will trial the efficacy of tarp collection of fresh feces and urine as a way of collecting viral dynamics data while reducing roost disturbance (REFS). To identify seasonal or reproductive cycle variation in viral dynamics, we will conduct repeated sampling of individuals and of tarps placed under the same roost site portion of a cave and examine roost-site fidelity (see below) to measure how well tarp-collected samples will track the general population. *Rhinolophus* species have a 7-week gestation period and generally give birth in the spring. Colony composition may change over the year, with bats aggregating during mating periods. These changes will affect viral dynamics and our sampling strategy will allow us to collect data over two mating and gestation periods and assess changes in viral prevalence. Additionally, we will conduct **pre-intervention (3 months prior to deployment) and post-intervention (3 months following deployment) CoV monitoring from these sites in Phase II (see Fig. X -Gantt chart)** to assess efficacy of our field intervention deployment. During months without physical bat trapping (2 months each quarter of sampling), fresh fecal pellets will be collected by placing clean polyethylene sheets measuring 2.0m x 2.0m beneath roosting bats. We will use infrared spotlights and digital infrared imaging to record the number and species of individuals above each plastic sheet. Fecal pellets may also be genetically barcoded to confirm species identification[43] as we routinely do for other bat surveillance projects. All specimens will be preserved in viral transport medium and immediately frozen in liquid nitrogen dry shippers in the field, then transported to partner laboratories with maintained cold chain and strict adherence to biosafety protocols. Each bat will be marked with a subcutaneous microchip (PIT tag) containing a unique ID number (**see below**). **Study caves and bat roosts will be surveyed using portable**

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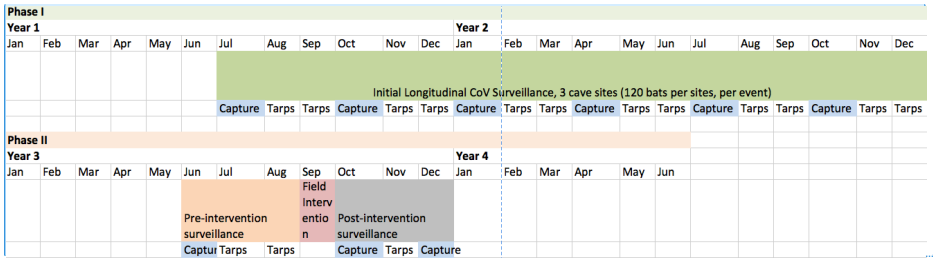
LiDAR technology[44-46], to give a 3-D image of the roost area which will provide data on species composition and volume/surface area that needs to be covered when applying the immune treatments in TA2 (Fig. XX). We will adjust individual sampling quotas per species to optimize viral detection based on host-specific prevalence of previous and ongoing host-pathogen models, as well as ongoing lab results from bat sampling.

Our team has more than 30 years of collective experience in safe and humane handling of bats for biological sampling. This project will operate under appropriate IACUC/ACURO and PPE guidelines. EHA has several ongoing DTRA-supported projects and is familiar with the process of obtaining ACURO approval for animal research from the DoD. The EHA team also currently maintains IACUC protocols through Tufts University (via inter-institutional agreement) and will obtain IACUC approval through this mechanism for DEFUSE.



Bats are highly mobile and little is known of inter-cave migration/emigration rates. To **monitor bat roost fidelity and movement** we will mark *Rhinolophid* bats with individual Passive Integrated Transponder (PIT) tags to track individual bats' entry and exit from roost caves. Tags will be inserted subcutaneously between the bats' scapulae by trained personnel. The identities of individually tagged bats inhabiting roost caves will be recorded using radio frequency identification (RFID) data loggers and antennae at the roost entrances. Time-stamped data from individual bats collected by data loggers will be downloaded every 3 days to examine temporal roost site fidelity and rates of inter-cave immigration/emigration. Infrared video cameras will record the total number of bats flying out each night. Recapture data will be collected continuously throughout the project. We will attach radio transmitters (1.2g, Advanced Telemetry Systems, MN USA), to the back of 20 individual *Rhinolophus sinicus* and *Rhinolophus ferrumequinum* from each study roost (60 total) to determine nightly foraging patterns and local dispersal patterns. Telemetry data and PIT tag data will be used to calculate home range, to determine the degree of mixing among our three sites, and parameterize our dynamic models. We will use fine scale data on roost fidelity to determine the population mix at the specific roost sites (e.g. a side pocket of a cave where only one species roosts) for our intervention. Radio transmitters that weigh <3% of bat body weight will be attached to the fur

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on the back using a veterinary dermatological adhesive (Vet Bond 3M, USA). We will collect location data from 60 bats (30 males, 30 females) every day for 10 days, 3 times per year for the 18 months of Phase 1. This will provide seasonal data to assess movement, including mating and gestation periods when higher levels of mixing and aggregation in the caves are expected.

High-risk SARSr-CoV quasispecies discovery, isolation and S. gene characterization. We will screen samples for SARSr-CoV nucleic acid using our pan-coronavirus consensus one-step hemi-nested RT-PCR (Invitrogen) assay targeting a 440-nt fragment in the RNA-dependent RNA polymerase gene (RdRp) of all known alpha- and betacoronaviruses assay[47,48], as well as specific assays for known SARSr-CoVs[27-30]. PCR products will be gel purified and sequenced with an ABI Prism 3730 DNA analyzer and quantitative PCR will be performed on SARSr-CoV-positive samples to determine viral load. Full-length genome of all detected SARSr-CoVs will be sequenced by high throughput sequencing method followed by genome walking. The sequencing libraries are constructed using NEBNext Ultra II DNA Library Prep Kit for Illumina and sequenced on a MiSeq sequencer, with PCR and Sanger sequencing used to fill gaps in the genome[29,30,32]. We will build phylogenetic trees using the Maximum Likelihood algorithm in the PhyML software, then scan for recombination events using Recombination Detection Program (RDP), confirmed using similarity plot and bootscan analyses in Simplot. We will analyze the S gene (which encodes the spike protein and determines receptor binding and cross-species transmission) of each sequence to identify a virus' potential to use human molecule ACE2 as a receptor. SARSr-CoVs with high similarity with SARS-CoV in full-length genomic sequences or with S proteins likely able to use human ACE2 as receptor will be identified as potential high-risk strains. We will then attempt isolation, cell culture, and infectious clone construction for further study *in vivo* and *in vitro* analysis. We have had success isolating and culturing SARSr-CoVs using Vero E6 monolayers in DMEM medium with 10% FCS, confirmed by RT-PCR and electron microscopy[29]. For SARSr-CoVs which we are not able to culture, we will construct recombinant viruses with the S gene of new bat SARSr-CoVs and the backbone of the infectious clone of SARSr-CoV WIV1 or of SARS-CoV, using the reverse genetic system described previously, and detailed below[28]. Initial assays of receptor usage and cell tropism will use various cell lines expressing human ACE2 incubated with isolated bat SARSr-CoVs or pseudotype viruses as previously shown[29].

Approach to predicting bat SARSr-CoV spillover risk. Our approach is to combine state-of-the-art genotype-phenotype modeling with detailed step-wise experimental characterization of each bat SARSr-CoV we identify at our test cave sites.
Flow chart here:
Sample testing/screening/Isolation – phylogenetic analysis/ACE2 binding modeling – ACE2 binding assays (all from Fig A) – chimera production – mouse model – SARS vaccines protect -

cross neut humAB – full length recovery (all from Fig b)-) – Data into predictive modeling (additional box)

This flow chart should use some elements of Ralph's figures A and B as indicated. Ask Ralph to send you Figs A and B in editable format so you can fuse them in the way above (a chimera!), and without the text. The flow chart needs to have less detail so the flow is visible when shrunk down.

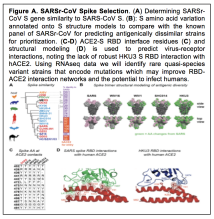

Figure A. SARSr-CoV Spike Selection. (**A**) Determining SARSr-CoV S gene similarity to SARS-CoV S. (**B**) S amino acid variation annotated onto S structure models to compare with the known panel of SARSr-CoV for predicting antigenically dissimilar strains for prioritization. (**C-D**) ACE2-S RBD interface residues (**C**) and structural modeling (**D**) is used to predict virus-receptor interactions, noting the lack of robust HKU3 S RBD interaction with hACE2. Using RNAseq data we will identify rare quasi-species variant strains that encode mutations which may improve RBD-ACE2 interaction networks and the potential to infect humans.

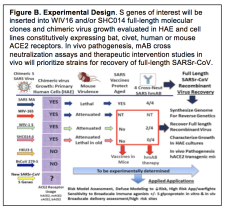

Figure B. Experimental Design. S genes of interest will be inserted into WIV16 and/or SHC014 full-length molecular clones and chimeric virus growth evaluated in HAE and cell lines constitutively expressing bat, civet, human or mouse ACE2 receptors. In vivo pathogenesis, mAB cross neutralization assays and therapeutic intervention studies in vivo will prioritize strains for recovery of full-length SARSr-CoV.

Our models will be parameterized with the experimental data from a series of assays on the S genes of bat SARSr-CoVs, with experimental and modeling work flowing together in iterative steps. The Baric laboratory pioneered many of the experimental approaches, the SARSr-CoV reverse genetic platforms, and full length S chimeric recombinant virus recovery from in silico sequence databases[7,8,23,49]. Full length recombinant strains reconstructed using reverse genetics in our lab include human epidemic strains, civet and raccoon dog SARS-CoV strains, and bat SARSr-CoVs (WIV16, WIV1, SHC014 and HKU3-SRBD repaired RBD interface). These strains will be used in the Baric, Shi and Wang laboratories for initial work on immune boosting and priming, and act as baseline data to parameterize the spillover risk modeling[7,8,23,49]. They will be supplemented by viruses we isolate under DEFUSE (worked on in the Shi lab) **and approximately 15-20 bat SARSr-CoV spike proteins/year** from DEFUSE (Baric, Shi labs). Most of the ~150 bat SARSr-CoV strains sequenced by us in prior work have not yet been examined for spillover potential and these will also be assessed in the following pipeline:

Experimental assays of SARSr-CoV spillover potential: *Ability to enter human cells:* Viral entry represents the key first step to evaluating the disease potential of SARSr-CoVs, with CoV species-specific restriction occurring primarily at entry[23,49]. To assess this we first will use structural modeling of SARSr-CoV S protein to ACE2 receptors. The structure of the SARS trimer prefusion S and the bound SARS-CoV S RBD to human and civet ACE2 have been solved, providing a platform for structural modeling and mapping hot spots of antigenic variation[50,51]. Mutations in the RBD[23,49,52,53], and host proteases and S glycoprotein proteolytic processing[54-56], regulate SARSr-CoV cell entry and cross-species infectivity. Mismatches in the S-RBD-ACE2 molecules or S proteolytic processing will prevent cell entry of SARS-CoV[23,49]. We will also conduct *in vitro* pseudovirus binding assays, as we have done previously for WIV1 and others[29],

as well as live virus binding assays for strains we are able to isolate. This work will be done in China (Shi lab), to prevent delays and unnecessary dissemination of viral cultures.
Novel SARSr-CoV Virus Recovery: We will commercially synthesize select SARSr-CoV S glycoprotein genes, designed for insertion into our SHC014 or WIV16 molecular clone backbones (these viruses are 88% and 97% identical to epidemic SARS-Urbani in the S glycoprotein). These are BSL-3, not select agents, and pathogenic in hACE2 transgenic mice. Different backbone strains provide increased opportunities for recovery of viable viruses, and to identify potential barriers for RNA recombination-mediated gene transfer between strains[30]. Chimeric viruses will be recovered in Vero cells, or in mouse cells over-expressing human, bat or civet ACE2 receptors to support cultivation of viruses with a weaker RBD-human ACE2 interface. All chimeric viruses will be sequence verified and evaluated for: **i**) human, civet and bat ACE2 receptor usage *in vitro*, **ii**) growth in primary HAE, **iii**) sensitivity to broadly cross neutralizing human monoclonal antibodies (mAB) S215.17, S109.8, S227.14 and S230.15 and a mouse antibody (435) that recognize unique epitopes in the RBD[57,58] and **iv**) *in vivo* pathogenesis studies in hACE2 transgenic mice, using our well established approaches[7]. Should some isolates prove highly resistant to our mAB panel, we will evaluate cross neutralization against a limited number of human SARS-CoV serum samples from the Toronto outbreak in 2003 (n=10). Chimeric viruses that encode novel S genes with spillover potential (e.g. growth in HAE, use of multiple species ACE2 receptor for entry, antigenic variation) will be used to identify SARSr-CoV strains for recovery as full genome length viable viruses. *Recovery of Full length SARSr-CoV:* We will compile sequence/RNAseq data from a panel of closely related strains (e.g.<5% nucleotide variation) and compare the full length genomes, scanning for unique SNPs representing sequencing errors[59-61]. The genome of consensus candidates will be synthesized commercially (e.g. BioBasic), as six contiguous cDNA pieces linked by unique restriction endonuclease sites for full length genome assembly. Full length genomes will be transcribed into genome-length RNA and electroporation used to recover recombinant viruses[22,62]. We will re-evaluate virus growth in primary HAE cultures at low and high multiplicity of infections and *in vivo* in hACE2 transgenic mice, testing whether backbone genome sequence alters full length SARSr-CoV spillover potential. All experiments will be performed in triplicate and data provided to the Modeling Team in real time. We anticipate recovering ~3-5 full length genomes/yr, reflecting strain differences in antigenicity, receptor usage, growth in human cells and pathogenesis. *In vivo Pathogenesis:* We generated a mouse that expresses human ACE2 receptor under control of HFH4, a lung ciliated epithelial cell promoter[7]. Infection of this model with wildtype SARS-CoV results in lethal disease, but transient disease with bat SARSr-CoV WIV1, suggesting that WIV1 is less efficient at using hACE2 *in vivo* and less likely to produce severe disease in people initially on spillover. However, single amino acid variations in the SARS-CoV RBD of related strains could dramatically alter these phenotypes, hence we will evaluate the impact of low abundant, high consequence

micro-variation in the RBD. Groups of 10 animals will be infected intranasally with 1.0 x 10^4 PFU of each vSARSr-CoV, then clinical disease (weight loss, respiratory function by whole body plethysmography, mortality, etc.) followed for 6 days p.i.. Animals will be sacrificed at day 2 or 6 p.i. for virologic analysis, histopathology and immunohistochemistry of the lung and for 22-parameter complete blood count (CBC) and bronchiolar alveolar lavage (BAL) using the Vetscan HM5 (an instrument that measures parameters used for human clinical determination). *Identification of high risk/low abundant variants:* We will use RNAseq to identify low abundant quasispecies (QS) variants encoding mutations in RBD and/or residues that bind ACE2. These would alter risk assessment calculations as strains identified as low risk, might actually have low abundant, high risk variants circulating in the QS. To test this the Shi and Baric lab will structurally model and identify highly variable residue changes in the SARSr-CoV S RBD and use commercial gene blocks to introduce these changes singly and then in combination into the S glycoprotein gene of the low risk, highly abundant parental strain. We will examine the capacity of these low abundance chimeric viruses to use human, bat, civet and mouse ACE2 receptors, and to replicate in HAE cultures. *RBD deletions:* Small deletions at specific sites in the SARSr-CoV RBD leave the key RBD-ACE2 interface residues intact, such that Clade 1 strains represent higher risk of human infection (Fig. 5). We will analyze the functional consequences of these RBD deletions on SARSr-CoV hACE2 receptor usage, growth in HAE cultures and *in vivo* pathogenesis. First, we will delete these regions, sequentially and then in combination, in SHC014 and SARS-CoV Urbani, anticipating that the introduction of both deletions will prevent virus growth in Vero cells and HAE. We hypothesize that the smaller deletion may be tolerated, given its location in the RBD structure, so *in vivo* passage in the presence of receptor will restore growth, while identifying 2nd site reversions that restore efficient hACE2 usage[49]. In parallel, we will evaluate whether RBD deletion repair restores the ability of low risk strains to use human ACE2 and grow in human cells. To test this we will synthesize full length rs4237, a highly variable SARSr-CoV that encodes a few of the SHC014 RBD contact interface residues but also encodes a mutation at 479 (N479S) and has two deletions and hence, is not recoverable *in vitro*. Using the SHC014 backbone sequence, we will sequentially and then in tandem repair the deletions in the presence and absence of the S479N. We anticipate that the S479N mutation is critical given its key role in establishing the RBD-ACE2 interface, and that restoration of the RBD deletions will enhance virus recognition of hACE2 receptors and growth in Vero cells and HAE cultures *S2 Proteolytic Cleave and Glycosylation Sites:* After receptor binding, a variety of cell surface or

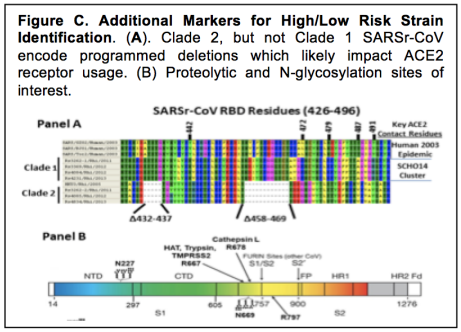

Figure C. Additional Markers for High/Low Risk Strain Identification. (**A**). Clade 2, but not Clade 1 SARSr-CoV encode programmed deletions which likely impact ACE2 receptor usage. (B) Proteolytic and N-glycosylation sites of interest.

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endosomal proteases[63-66] cleave the SARS-CoV S glycoprotein causing massive changes in S structure [67] and activating fusion-mediated entry[55], which is prevented in the absence of S cleavage[68] (Fig. 5). Tissue culture adaptations sometimes introduce a furin cleavage site which can direct entry processes, usually by cleaving S at positions 757 and 900 in S2 of other CoV, but not SARS[66]. For SARS-CoV, a variety of key cleavage sites in S have also been identified and we will analyze all SARSr-CoV S gene sequences for appropriately conserved proteolytic cleavage sites in S2 and for the presence of potential furin cleavage sites[69,70]. SARSr-CoV S with mismatches in proteolytic cleavage sites can be activated by exogenous trypsin or cathepsin L. Where clear mismatches occur, we will introduce the appropriate human-specific cleavage sites and evaluate growth potential in Vero cells and HAE cultures. In SARS-CoV, we will ablate several of these sites based on pseudotyped particle studies and evaluate the impact of select SARSr-CoV S changes on virus replication and pathogenesis (e.g. R667, R678, R797). We will also review deep sequence data for low abundant high risk SARSr-CoV that encode functional proteolytic cleavage sites, and if so, introduce these changes into the appropriate high abundant, low risk parental strain. *N-linked glycosylation:* SARS-CoV S has 23 potential N-linked glycosylation sites and 13 of these have been confirmed biochemically. Several of these regulate SARS-CoV particle binding DC-SIGN/L-SIGN, alternative entry receptors for SARS-CoV entry into macrophages/monocytes[71,72]. Mutations that introduced two new N-linked glycosylation sites may have been involved in the emergence of human SARS-CoV from civet and raccoon dogs[72]. While the sites are absent from civet and raccoon dog strains as well as clade 2 SARSr-CoV, they are present in WIV1, WIV16 and SHC014, supporting a potential role for these sites in host jumping. To evaluate this, we will sequentially introduce clade 2 residues at positions N227 and N699 of SARS-CoV and SHC014 and evaluate virus growth in Vero cells, nonpermissive cells ectopically expressing DC-SIGN and in HAE cultures, as well as in human monocytes and macrophages anticipating reduced virus growth efficiency. Using the clade 2 rs4237 molecular clone, we will introduce the clade I mutations that result in N-linked glycosylation sites at positions 227 and N699 and in rs4237 RBD deletion repaired strains, evaluating virus growth efficiency in HAE, Vero cells, or nonpermissive cells ± ectopic DC-SIGN expression[72]. *In vivo*, we will evaluate pathogenesis in transgenic ACE2 mice.

Models to predict viral spillover potential and evolution of high-risk SARSr-CoV strains. *Structural equation model of spillover potential:* We will use data from the experimental assays above to **build genotype-phenotype models of bat SARSr-CoV spillover potential**. We will use Bayesian Structural Equation Models (SEM), fit via MCMC methods[73], to predict spillover potential from the genetic traits of bat SARSr-CoVs and the ecological traits of hosts. SEMs have successfully analyzed the drivers of, and predicted stochastic species interactions[74,75]. They will enable us to integrate multiple, interrelated tests of strain spillover potential into a common framework, while restricting relationships to plausible causal pathways. This prevents the over-

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fitting associated with a black-box approach. A Bayesian approach allows fitting with unbalanced and non-independent data, as per the larger number of cell-binding and cell-entry assays we will run to determine candidates for a smaller number of humanized mouse trials and LIPS assays (below). The viral traits derived from the experimental assays of spillover risk laid out above will be our primary set of predictor variables: presence of deletions in the RBD region, proteolytic binding sites, glycosylation sites, neutralization escape mutations, indeterminate mutations at high-variation sites found in low-abundance strains. We will include genetic similarity of each strain's RBD to the reference pandemic SARS-CoV genomes to test these aggregate measures as predictive proxies. To control for experimental conditions we will include whether assays were performed on live viral isolates, full-genome or synthetic chimeric viruses, and the molecular backbone used in the latter. These traits will be used as inputs to SEM's causal graph, and used to predict latent variables representing the interconnected processes that contribute to SARSr-CoV QS spillover potential: receptor binding, cell entry with and without the presence of exogenous proteases, immune system interaction, and intracellular growth, all measured by our laboratory assay. These, in turn will **act as predictors for the ultimate outcomes of host pathogenesis (Fig. 6)**. We will use previous work on these genetic traits to put informative priors on strength and direction of interactions in the causal graph. We will use **prior-knowledge model simulations to select target sequences from our sampling for characterization and genome-sequencing**, to collect data that maximally enhances the predictive power of our model. We will use regularizing priors to reduce over-fitting and help select the most predictive variables in the final predictive model.

Evolutionary modeling and simulation to predict potential strains: Our SEM modeling will generate estimates of the spillover potential of SARSr-CoV sequences from DEFUSE fieldwork and prior work. To examine risk associated with the total viral population at our test sites, we will model and simulate evolutionary processes **to identify likely viral QS that our sampling has not captured, as well as viral QS likely to arise in the future**. By estimating the spillover potential of these simulated QS, we can better characterize the risk associated with the total viral population. We will use a large dataset of S protein sequences and full-length genomes generated from prior work and DEFUSE fieldwork to estimate SARSr-CoV substitution rate and its genome-wide variation using coalescent and molecular clock models within a Bayesian MCMC framework[76]. We will then estimate SARSr-CoV recombination rates at the cave population level using the same dataset and Bayesian inference[77,78]. We will apply various methods (RDP[79], similarity plots, bootscan) to identify recombination breakpoints and hotspots within the SARSr-CoV genome. Using these estimates of substitution and recombination rates, we will simulate the evolution of the SARSr-CoV QS virome using a forward-time approach implemented in simulators that model specific RNA virus functions (e.g. VIRAPOPS[80]). This will allow us to predict the rate at which new combinations of genetic traits can spread in viral populations and compare recombination rates among caves and bat communities. Our forward-

simulated results **will provide a pool of likely unknown and future QS species**. Using these and our SEM model for spillover risk, **we will predict the QS that are most likely to arise *and* have pathogenetic and spillover potential.** We will use the evolutionary simulation results to iteratively improve our SEM model results. The number of genetic traits of interest for prediction of pathogenicity is potentially large, so we will perform variable reduction using tree-based clustering, treating highly co-occurring traits as joint clusters for purposes of prediction. We will generate these clusters from our full set of SARSr-COV sequences from DEFUSE fieldwork and prior work. However, as trait clusters may be modified in future virus evolution due to recombination, we will use our forward-evolutionary modeling to predict how well trait clusters will be conserved, retaining only those trait clusters unlikely to arise in unknown or future viral QS genomes. This will enable a good trade-off between increased predictive power based on current samples and generalizability to future strains that have not yet evolved.

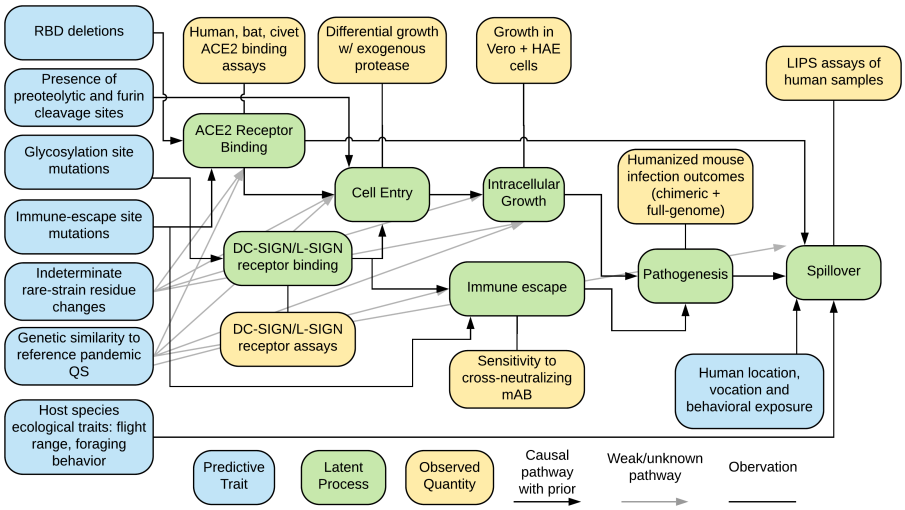

Figure 6: A simplified directed graph of a structural equation model representing the causal relationships between predictors and measures of viral pandemic potential.

Validation by LIPS assay on previously-collected human sera: Following our proof-of-concept field trial we will update these models to include not only pathogenesis but spillover probability validated with data on viral QS antibodies found in the local human population detected via Luciferase immunoprecipitation system (LIPS) assays on previously-collected human sera (NIAID project, Daszak PI). This includes >2,000 samples collected from people living close to our test cave sites in Yunnan Province, and is the basis of a recent paper demonstrating 2.7% seropositivity to bat SARSr-CoVs in an initial sampling of this population[34] (Fig. 7). In addition to

serum samples, extensive behavioral and wildlife contact data has been collected from this population, under an IRB that can be easily extended to cover DEFUSE work.

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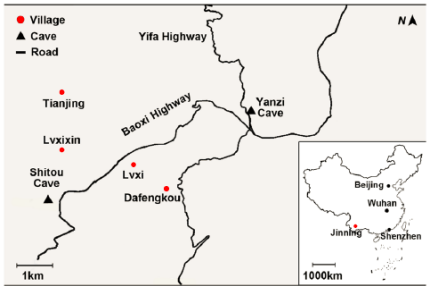

Fig. 7. Human sera were collection from villages (red dots) near bat caves where CoV positive samples have been isolated (Yanzi Cave and Shitou Cave, triangle).

Our ability to extend and validate these models with data on actual human contact and spillover allows us to fit and test models of actual, not just potential, spillover probability. Our previous work has shown that both host and viral traits predict zoonotic spillover from models[3], so in addition to viral traits, we will include key ecological traits of the host bat species in which viral QS were detected. These include flight ranges, foraging, roosting, demographic, and social behavior. To will use the extensive data on each person's behavioral exposure to wildlife, and their work, travel and occupational history, to correct for varying human exposure to bat species. We will design LIPS assays for specific high- and low-spillover risk SARSr-CoVs, to identify people who've been exposed to them, and test our model's validity. The LIPS uses viral antigens tagged with luciferase, from crude lysate, thereby eliminating the requirement for antigen purification and significantly reducing the time required for assay development and producing a more sensitive test than traditional ELISA[81]. Prof. Zhengli Shi (Wuhan Institute of Virology) will lead the LIPS serological work based on her 15 years SARSr-CoV human serological surveillance experience [82-84] and the recent success in SADS-CoV zoonotic risk study using LIPS[85]. To establish SARSr-CoV LIPS assays, we will: **1)** Insert different high- and low-risk SARSr-CoV N genes into pREN-2 vector (LIPS vector). We will first assess N gene similarity to determination their potential cross-reactivity in a LIPS assay. From our previous experience, SARSr-CoV maintain 80% similarity in the N protein, thus should be detectable using a universal SARSr-CoV N based LIPS assay; **2)** determine specificity of the LIPS assay by producing polyclonal sera via injection of recombinant protein or attenuated virus into rabbits. Selected SARSr-CoV N proteins or viral particles will be used as the immunogen for antibody production; **3)** validate SARS-CoV, MERS-CoV and SADS-CoV N protein LIPS assays by incubating antigens with their respective positive serum samples and the antigen antibody complex eluted using protein A/G beads. Luminescence is measured upon adding coelentrazine, a substrate of renilla luciferase. In a preliminary assay, LIPS successfully detected high strong antibody titer in the positive control serum sample, while the vector control did not show any response. Cut off was set as the average luminescence plus three standard deviation from the control. We have used this to demonstrate efficacy for MERS-CoV and SADS-CoV (Fig. 8); **4)** validate LIPS positive sera results by spike protein based

LIPS and viral neutralization assay. Similarly, S gene from high/low risk SARSr-CoV will be engineered into the pREN-2 vector and an S-LIPS assay produced, as above. As a confirmatory test the positive samples from LIPS, will be validated by viral neutralization assay. The data from LIPS and neutralization will be collected and analysis to validate the model.

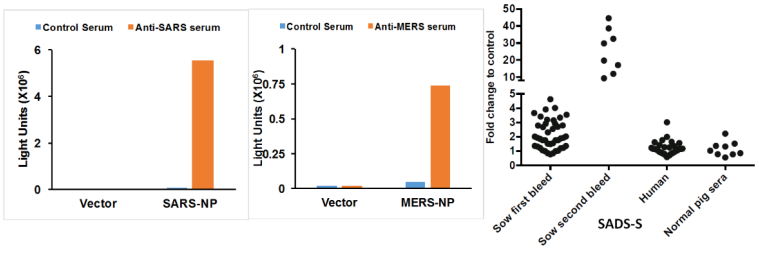

Fig. 8. LIPS assay was tested successful for SARS, MERS and SADS coronavirus N or S antibodies.

Thematic Area 2

Immune modulation approach to reducing bat SARSr-CoV spillover risk. There is no available technology to reduce the risk of exposure to novel CoVs from bats which carry zoonotic precursors to many emerging viruses including filoviruses (Ebola), CoV (SARS-CoV, MERS-CoV, etc.), paramyxoviruses (Nipah/Hendra), rhabdoviruses (rabies) and others. No vaccines or therapeutics exist for emerging CoVs, filoviruses and paramyxoviruses and exposure mitigation strategies are non-existent. We have shown that bats have unique immunological features that may explain why they coexist with viruses and rarely show clinical signs of infection. Our long-term studies demonstrate: a) bats maintain constitutively high expression of IFNα that may respond to and thus restrict, viral infection immediately[11]; b) several bat interferon activation pathways are dampened, e.g. STING (a central cytosolic DNA-sensor molecule to induce interferon) dependent and TLR7 dependent pathways[10]; c) the NLRP3 dependent inflammasome pathway is dampened, and some of the key inflammation response genes like AIM2 have been lost in bats[86,87]. The dampened IFN and inflammasome response suggest bats maintain a fine balance between IFN response and detrimental over-response. This is likely due to an adaptation of their immune-sensing pathways as a fitness cost of flight[9]. We hypothesize that the bat innate/adaptive immune responses are quite different from that of human and mouse. Firstly, virus replication will likely be restricted quickly by constitutively expressed IFNα in bats, resulting in lower B/T cell stimulation due to lower viral stimuli. Second, dampened interferon and inflammasome responses will result in lower cytokine responses that are required to trigger T/B cell dependent adaptive immunity (e.g. antibody response). The strong innate immune response, due to the lack of an efficient antibody response, will clear the virus.

We and others have demonstrated proof-of-concept of this phenomenon: Experimental Marburg virus infection of Egyptian fruits bats, a natural reservoir host, resulted in wide tissue distribution yet low to moderate viral loads, brief viremia, low seroconversion and a low antibody titer that waned quickly, suggesting no long-term protection is established[88-90]. Similarly, poor neutralizing antibody responses occur after experimental infection of bats with Tacaribe virus[91] and in our studies with SARS-CoV experimentally infected bats (L-F Wang, unpublished data). Indeed, we successfully showed bat interferon can inhibit bat SARSr-CoVs[28]. We hypothesize that if we can use immune modulators that upregulate the naturally low innate immunity of bats to their viruses, we will be able to transiently suppress viral replication and shedding, reducing the risk of spillover. We will evaluate two immune modulation approaches to defuse spillover of SARSr-CoVs from bats to humans: **1) Broadscale Immune Boosting strategies (Wang, Duke-NUS):** we will apply immune modulators like TLR-ligands, small molecule Rig like receptor (RLR) agonists or bat interferon in live bats, to up-regulate their innate immunity and assess suppression of viral replication and shedding; **2) Targeted Immune Priming (Baric, UNC):** the broadscale immune boosting approach will be applied in the presence and absence of chimeric immunogens to boost clearance of high-risk SARSr-CoVs. Building on preliminary development of polyvalent chimeric recombinant SARSr-CoV spike proteins, we will use novel chimeric polyvalent recombinant S proteins in microparticle encapsidated gels and powders for oral delivery and/or virus adjuvanted immune boosting strategies where chimeric recombinant SARSr-CoV S are expressed from raccoon poxvirus, which has been used extensively to deliver rabies immunogens in bats and other animals. We will conduct application trials with live bats to assess suppression of replication and shedding of a broad range of pathogenic SARS-related CoVs. Both lines of work will begin in Year 1 and run parallel, be assessed competitively for efficiency, cost, and scalability, and successful candidates used in our live bat trials at our test sites in Yunnan, China. We believe an immune boosting/priming strategy is a superior approach for this challenge because solutions are likely to be broadly applicable to many bat species, and across many viral families.

Broadscale immune boosting (led by Wang, Duke-NUS). We will work on the following key leads to identify the most effective approach to up-regulate innate immunity an suppress viral loads. *Toll-like receptor (TLR)/Rig-I Like Receptor (RLR) ligands:* We have begun profiling bat innate immune activation *in vivo*, in response to various stimuli. Our work indicates a robust response to TLR-stimuli like polyI:C when delivered *in vivo*, as measured by transcriptomics on spleen tissue (Fig. 7). We have performed transcriptomics on spleen, liver, lung and lymph node, with matched proteomics to characterize immune activation *in vivo*. These activation profiles will be used to assess the bat immune response to different stimuli and direct the response to favor those which lower the viral load in our experimental system at Duke-NUS (below). In addition to the ligands already tested, we will stimulate the Rig-I pathway with

5'pppDSRNA, a mimetic of the natural RIG-I stimulant. These stimulants will activate functional bat IFN production pathways, and a similar strategy has been demonstrated in a mouse model for clearance of SARS-CoV, influenza A virus and Hepatitis B virus[12,15].

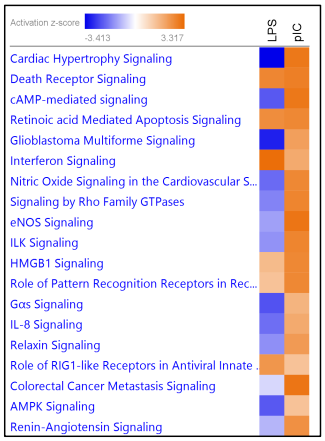

Fig. 7. Pathway analyses from Ingenuity Pathway Analysis (IPA) of whole spleen NGS after stimulation with either LPS or polyI:C. Z-score increase over control bats is indicated as per scale, and suggests strong activation of many pathways.

Universal bat interferon: To overcome any complications arising from species-specificity, we will design a conserved universal bat interferon protein sequence and produce purified protein. Utilization of a universal IFN for bats will overcome species-dependent response to the ligand, allowing the use of IFN throughout broad geographical and ecological environments and across many bat species. As a starting point, we have produced recombinant non-universal, tagged, bat IFN that are effective at inducing appropriate immune activation (Fig. 8). This ligand can be **delivered by aerosol or intranasal application** as has been shown to reduce viral titers in humans, ferrets and mouse models[12,13,15]. Interferon has been used clinically in humans as an effective countermeasure when antiviral drugs are unavailable, e.g. against filoviruses[14]. Replication of SARSr-CoV is sensitive to IFN treatments, as shown in our previous work[28]. The successful delivery, immune activation and outcome on the host will be characterized thoroughly to optimize rapid immune activation.

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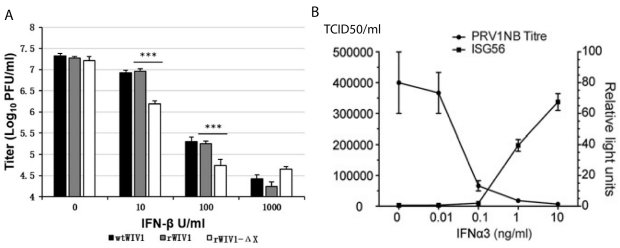

Fig. 8: Bat viruses are sensitive to IFN treatments. A) Recombinant bat SARS-related coronavirus WIV1 replication was inhibited by human IFN-β in a dose dependent manner in Vero cells. B) Bat reovirus PRV1NB replication was inhibited by recombinant bat IFNα3 in a dose dependent manner in bat PakiT03 cells.

Boosting bat IFN by blocking bat-specific IFN negative regulators: Uniquely, bat IFNα is naturally constitutively expressed but cannot be induced to a high level, indicating a negative regulatory factor in the bat interferon production pathway[92]. To fast-track the identification of this target we will utilize a *Pteropus alecto* CRISPRi library pool that we have created covering multiple RNA targets in every gene in the *P. alecto* genome. The library has already been produced and

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genes affecting influenza replication in bat cells have been identified. Using CRISPRi we can identify negative regulator genes and then screen for compounds targeting these genes to boost the inducibility of the IFN system in a shorter time-frame. Based on previous work, it is highly likely this will be a conserved pathway throughout the order *Chiroptera*. *Activating dampened bat-specific innate immune pathways which include DNA-STING-dependent and TLR-dependent pathways:* Our work showing that mutant bat STING or reconstitution of AIM2 and functional NLRP3 homologs restores antiviral functionality suggests these pathways are important in bat-viral coexistence and that the majority of the pathway is preserved. By identifying small molecules to directly activate pathways downstream of STING or TLR/RLRs, such as TBK1 activation, we will activate bat innate defense by interferons and promote viral clearance. We hypothesize that these small molecules we will be able to significantly reduce viral load in bats. *Validation in a bat-mouse model.* Various CoVs show efficient infection and replication inside the human host but exhibit defective entry and replication using mouse as a host due in part to differences in DPP3 and ACE2 receptors. We have shown efficient reconstitution of irradiated mice using bat bone marrow from multiple species, including *E. spelaea*. Fig. 9 shows the efficient reconstitution of bat PBMC's in the mouse, presence of circulating bat cells and generation of bat-specific antibodies in mice incapable of producing an antibody response. This 'batized' mouse model can be utilized for both circulating infection of SARS/MERS CoV (in the immune compartment only) and as a model for generating bat-specific antibodies against CoV proteins. Efficient validation of infection into bat cells will be used to validate the infectivity of the viruses and generation of bat antibodies will facilitate validation of the best proteins/peptide to elicit an effective immune response.

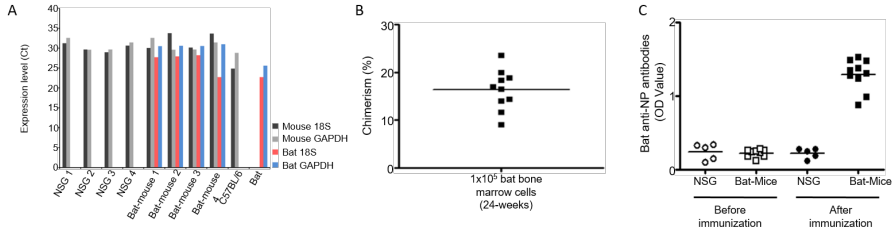

Fig. 9: A) Presence of bat-specific qPCR in reconstituted mice after 12 weeks. B) chimeric ratio of bat-mouse cells in circulation after 24 weeks. C) Specific antibody response to a KLH-tetanus antigen generated by bat-reconstituted mice.

Viral infection models in cave-nectar bat (Duke-NUS): To test and compare the efficacy of the immune modulating approaches above, we will use our cave-nectar bat (*Eonycteris spelaea*) breeding colony infected with Melaka virus (family *Reoviridae*) which is known to infect this species[93,94]. We will also use two coronaviruses (SARSr-CoV WIV1 and MERS-CoV in ABSL3.

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Details of infection, housing, prior infection trials in the facility… Viral loads will be measured by qPCR, titration of produced virus, NGS transcriptomics and nanostring probes added to the immunoprofiling panel. Antibody responses will be measured by LIPS assay. This approach allows us to test our immune-boosting strategies, in a safe and controlled environment, prior to expanding to field-based evaluation. The analytical methods used for the *E. spelaea* colony will be replicated to analyze the experimental infection of *Rhinolophus* in a wild-cave scenario. Additionally, the versatility of the analysis should allow easy application to multiple species of bats

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Targeted Immune Priming (led by Baric, UNC). We have developed novel group 2b SARSr-CoV chimeric S glycoproteins that encode neutralizing domains from phylogenetically distant strains (e.g. Urbani, HKU3, BtCoV 279), which differ by ~25%. The chimeric S programs efficient expression when introduced in the HKU3 backbone full length genome, and elicit protective immunity against multiple group 2b strains. We will develop robust expression systems for SARSr-CoV chimeric S using ectopic expression *in vitro*. Then, we will work with Dr. Ainslie (UNC-Pharmacy) who has developed **novel microparticle delivery systems and dry powders for aerosol release**, and which encapsidate recombinant proteins and adjuvants (innate immune agonists) that will be used for **parallel broadscale immune boosting strategies ± chimeric immungens.** Simultaneously, we will introduce chimeric and wildtype S in raccoon poxvirus (RCN), in collaboration with Dr. Rocke and confirm recombinant protein expression, first *in vitro* and then in the Duke-NUS bat colony, prior to any field trial. The goal of this aim is to develop a suite of reagents to remotely reduce exposure risk in high risk environmental settings.

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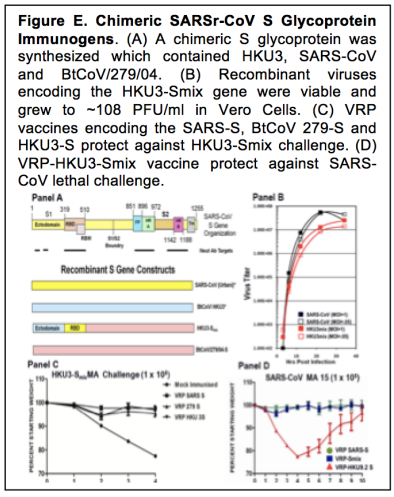

Figure E. Chimeric SARSr-CoV S Glycoprotein Immunogens. (A) A chimeric S glycoprotein was synthesized which contained HKU3, SARS-CoV and BtCoV/279/04. (B) Recombinant viruses encoding the HKU3-Smix gene were viable and grew to ~108 PFU/ml in Vero Cells. (C) VRP vaccines encoding the SARS-S, BtCoV 279-S and HKU3-S protect against HKU3-Smix challenge. (D) VRP-HKU3-Smix vaccine protect against SARS-CoV lethal challenge.

Chimeric SARSr-CoV S Immunogens: CoV evolve quickly by mutation and RNA recombination, the latter provides a strategy to rapidly exchange functional motifs within the S glycoprotein and generate viruses with novel properties in terms of host range and pathogenesis[30,95]. CoV also encode neutralizing epitopes in the amino terminal domain (NTD), RBD and S2 portion of the S glycoprotein[57,96,97], providing a strategy to build chimeric immunogens that induce broadly cross reactive neutralizing antibodies. Given the breadth of SARSr-CoV circulating in natural settings, chimeric immunogens will be designed to increase the breadth of neutralizing epitopes across the group 2b phylogenetic subgroup[40]. Using synthetic genomes and structure

guided design, we fused the NTD of HKU3 (1-319) with the SARS-CoV RBD (320-510) with the remaining BtCoV 279/04 S glycoprotein molecule (511-1255), introduced the chimeric S glycoprotein gene into the HKU3 genome backbone (25% different than SARS-CoV, clade 2 virus) and recovered viable viruses (HKU3-S_{mix}) that could replicate to titers of about 10^8 PFU/ml on Vero cells (Fig. 10). HKU3-Smix is fully neutralized by mAb that specifically target the SARS RBD (data not shown). In parallel, we inserted the $HKU3_{mix}$ S glycoprotein gene into VEE virus replicon vectors (VRP-$S_{chimera}$) and demonstrated that VRP vaccines protect against lethal SARS-CoV challenge and virus growth. In addition, VRP-S_{HKU3} and VRP-S_{279} both protect against $HKU3_{mix}$ challenge and growth *in vivo* (Fig. 9), demonstrating that neutralizing epitopes in the $HKU3_{mix}$ S glycoprotein are appropriately presented and provide broad cross protection against multiple SARSr-CoV strains. In addition to using these immunogens as a targeted broad-based boosting strategy in bats, we will also produce a chimeric SHC014/SARS-CoV/HKU3 S and a SCH014/SARS-CoV/WIV-1 S gene for more focused immune targeting on known high risk strains. In parallel, we will work with the Protein Expression Core at UNC (https://www.med.unc.edu/csb/pep) to produce codon optimized, stabilized and purified prefusion SARS-CoV glycoprotein ectodomains as published previously[17]. Purified recombinant protein will be used by Drs. Rocke and Ainslie for inclusion in delivery matrices (e.g. purified powders, dextran beads, gels – see below) with broadscale immune agonists (adjuvants-Dr. Wang) like poly IC, TLR4 and Sting agonists.

2nd Generation Chimeric S glycoprotein Design and Testing: We will also produce a chimeric SHC014 NTD/SARS-CoV-RBD/HKU3 S C terminal and generate recombinant HKU3 encoding the trimer spike (HKU3-S_{S014}), for more focused immune targeting on known high and low risk strains designated from our experimental and modeling analyses. A second construct will be synthesized with a SHC014 NTD domain, SARS-CoV RBD and WIV-1 C terminal domain (WIV-S_{S014}). After sequence variation, we will evaluate virus growth in Vero and HAE cultures and the ability of SARS RBD monoclonal antibodies (S227, S230, S109) to neutralize chimeric virus infectivity[89,96]. We will also evaluate *in vivo* pathogenesis in C57BL/6 mice and hACE2 transgenic mice. The recombinant HKU3-S_{S014} S genes will be introduced into VRP vectors and sent to Dr. Rocke for insertion into the raccoon poxvirus vaccine vector. Using established techniques, we will characterize S expression and then provide virus vectors to Prof. Wang for immune boosting trials at Duke-NUS, and ultimately if successful in the field (Prof. Shi). We will also synthesize human codon optimized the HKU3-S_{S014}, WIV-S_{S014} and HKU3-S_{mix} chimeric spikes for expression and purification by the UNC proteomics core, producing mg quantities for inclusion in nanoparticle and microparticle carriers in collaboration with Dr. Ainslie. We will produce enough material for *in vivo* testing in mice and in bats. Recombinant HKU3-S_{S014} and WIV-S_{S014} glycoprotein expression will be validated by Western blot and by vaccination of mice, allowing us to determine if the recombinant protein elicits neutralizing antibodies that protect against lethal SARS-CoV, HKU3-S_{mix} and SHC014 challenge. In parallel, we will survey the RNAseq data

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for evidence of complex S glycoprotein gene RNA recombinants in the SARSr-CoV population genetic structure. If present, we will synthesize 2-3 interesting recombinant S genes, insert these genes into SHCO14 or HKU3 genome backbones and VRP and characterize the viability and replicative properties of these viruses in cell culture and in mice and the VRP for S glycoprotein expression and vaccine outcomes. We will produce immunogens and evaluate their ability to protect against infection.

Adjuvant and Immunogen Delivery Vehicles. Dr. Ainslie (UNC) and collaborators have developed the biodegradable polymer acetalated dextran (Ac-DEX) for the delivery of antigens and adjuvants in vaccine applications (Fig. 11). Ac-DEX has distinct advantages over other polymers for vaccine development: 1) synthesis is straightforward and scalable. An FDA-approved water soluble dextran polysaccharide is modified and rendered insoluble in water by a simple one-step modification of its hydroxyl groups with pendant acyclic or cyclic acetal groups[98-100]. Unlike other dextran based vaccine materials, our material is acid sensitive, which has been shown to greatly improve antigen presentation; 2) Ac-DEX microparticles (MPs) can passively target antigen-presenting cells (APCs) based on their size (5-8μm), being phagocytosed by DCs and traffic to the lymph node[101]. Furthermore, APCs have acidic phagosomes that can result in triggered intracellular release due to the acid-sensitivity of Ace-DEX; 3) Ac-DEX MPs and their hydrolytic byproducts are pH-neutral, biocompatible, and safe compared to other commonly used polyesters have acidic hydrolytic byproducts (e.g. lactic and glycolic acid, in the case of PLGA) that damage vaccine components such as protein antigens[102]. The complete hydrolysis of Ac-DEX results in particle breakdown with release of the metabolic side products. 4) Ac-DEX MPs are stable outside the cold-chain. MPs can be stored for at least 3 months at 45°C without any loss of integrity or encapsulated cargo bioactivity[103]. Other common formulations (e.g. liposomes[104], PLGA MPs[103], squalene emulsions [Fluad™ package insert]) have limited shelf-life that requires the cold-chain. Ac-DEX MPs can be aerosolized, or delivered in sprays or gels to bat populations, providing new modalities for zoonotic virus disease control in wildlife populations[98,105]. 5) We have previously encapsulated Poly (I:C)(1), resiquimod[101], and a STING agonist into our novel MPs[106].

As seen in Fig. 10, encapsulation of Poly (I:C) drastically enhances the activity of the TLR agonist. Additionally, encapsulation of adjuvants in MPs drastically enhances the activity of subunit vaccines. We have displayed better efficacy than state-of-the-art FDA-approved inactivated flu virus (Fluarix) in a ferret model of influenza. The

Figure F. Particle Delivery Systems. Broadscale immune boosting strategies include (A) Dextran microparticles or Dry nanoparticle powders. (B) Macrophages cultured with either free poly (I:C) or poly (I:C) encapsulated into Ac-DEX MPs produce significant TNFα. (C) Comparison of (left) neutralizing titer and (right) viral load when ferrets are vaccinated with Ac-DEX MPs. Day 0, 28, and 56 (prime, boost, and challenge.)

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ferret model is the ideal animal model for influenza because of their relatively small size and they possess various clinical features associated with human influenza infection[107]. This formulation used HA with encapsulated STING agonist cyclic [G(3',5')pA(3',5')p](16) *Microparticle Performance Metrics in vitro and in Rodents and Bats:* MPs are designed for aerosol delivery due to their relatively effective low aerodynamic diameter[108], their low density microporous nature which allows for efficient aerosol dispersal and deep penetration into the lung, or deposition on the skin for oral uptake by grooming. We will encapsulate Poly (I:C), resiquimod (TLR 7) or other innate immune agonists to enhance type I interferon production in in consultation with Prof Wang. Agonist laden particles will be made separately or in combination with recombinant SARS-CoV chimeric spike proteins, encapsulated into our aerodynamic MPs as well as nanoparticles.

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Delivery system development (Rocke, NWHC). We have previously developed, tested and registered oral vaccines and delivery methods to manage disease in free-ranging wildlife including a sylvatic plague vaccine for prairie dogs[24], vaccines against bat rabies[25] and white-nose syndrome (unpubl. data). We have optimized vaccine delivery methods, uptake by the target species and safety in non-target hosts using biomarkers prior to deployment[109]. We will use a similar approach to develop, test and optimize delivery methods to *Rhinolophus* bats in SE Asia. While work on immune modulating agents progresses, we will concurrently develop and test mediums, routes, and methods of delivery to large colonies of bats. We will determine the most feasible and simple method of delivery that achieves high uptake by bats, is safe for humans as well as target and non-target species, and minimizes colony disturbance. Sticky edible gels or pastes that bats groom from themselves and each other have been used previously to deliver pharmaceuticals to bats orally and we are currently testing these for use in rabies vaccine delivery. These may also be useful for delivering immune modulators and recombinant SARSr-CoV spike proteins to *Rhinolophus* bats, but may need to be combined with viral vectors (like poxvirus or adenovirus) or nanoparticles/nanoemulsions that enhance uptake through mucous membranes or transdermally after topical application. *Poxvirus vectors:* Poxviruses are effective viral vectors for delivering vaccines to wildlife [24,110,111], and can replicate safely at high levels in bats after oronasal administration[26]. We have demonstrated proof-of-concept in bats. We modified vaccinia Ankara (MVA) and raccoon poxvirus (RCN) vecotrs for safety and replication in bats using *in vivo* biophotonic imaging[25]. RCN replicated to higher levels in bats than MVA, even via the oral route, and was found to be highly safe for bats (Fig. 12). We used raccoon poxvirus-vectored novel rabies glycoprotein (mosaic or MoG) and demonstrated protective efficacy in bats after oronasal and topical administration[25] (Fig. 13). We are currently developing vaccine delivery for vampire bats in several Latin American countries, and vaccines for white-nose syndrome in bats, a devastating disease that has killed millions of insectivorous bats in North America.

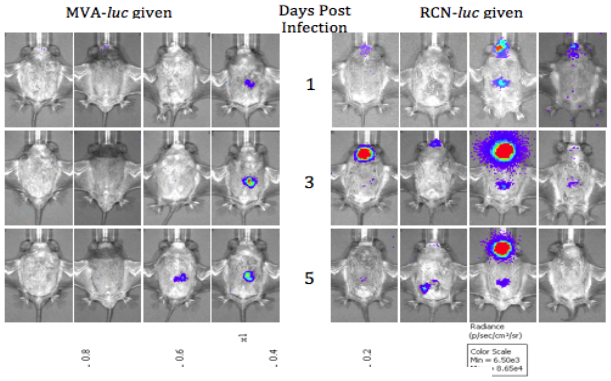

Fig. 12. Luminescence, indicative of viral replication of modified vaccinia Ankara (MVA) and raccoon poxvirus RCN) in the bat Tadarida brasiliensis *on 1, 3 and 5 dpi via the oronasal route.*

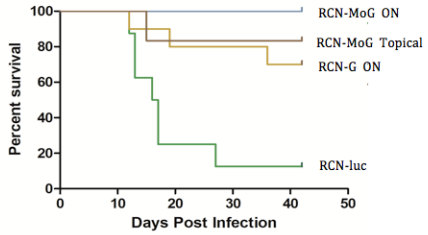

Figure 13. Vaccine efficacy and rabies challenge in Epstesicus fuscus immunized with raccoon poxvirus expressing a mosaic G protein (RCN-MoG) either oronasally (ON) or topically in comparison to RCN expressing typical G protein and RCN expressing luciferase (negative control).

Poxviruses are safe in a wide variety of wild and domestic animals, and allow for large inserts of foreign DNA. We have previously used a raccoon poxvirus vectored vaccine expressing plague antigens was incorporated into a peanut-butter flavored bait matrix to manage plague caused by *Yersinia pestis* in prairie dogs. We incorporated the biomarker Rhodamine B (RB) into baits to assess uptake by target and non-target species [109,112] (Fig. 14). RB is visible under a UV microscope until the hair grows out (~50 days in prairie dogs). We have since conducted a large field trial (approved by USDA Center for Veterinary Biologics) that demonstrated vaccine efficacy in four species of prairie dogs in seven western states[24]. We used biomarker analysis to assess site- and individual host-specific factors that increased bait consumption including age, weight, and the availability of green vegetation.

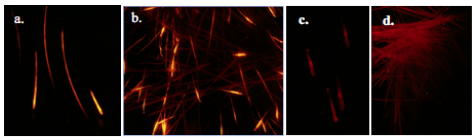

Fig. 14. Prairie dog hair and whisker samples under fluorescence microscope (excitation wavelength: 540 nm, emission wavelength: 625 nm) to determine uptake of baits containing Rhodamine B. a) 20 days after bait distribution, b) 16 days after bait distribution, c) and d) controls (note natural dull fluorescence).

Transcutaneous delivery: In addition to viral, we will also consider methods to achieve

transcutaneous delivery of the immune boosting proteins without the use of live agents. Nanoparticles have been used to increase transcutaneous delivery efficiency[113]. However, the impermeable stratum corneum provides a difficult barrier to breach. Mechanical approaches have been used[113] but are somewhat unethical and impractical for wildlife. We are currently testing poly lactic-co-glycolic acid (PLGA) as a nanoparticle to encapsulate rabies glycoprotein as a method of transcutaneous delivery of vaccine to bats via dendritic cell uptake[114], as has been shown for delivery of TLR agonists and antigens simultaneously to mice[115]. This approach will be competitively trialed against ac-DEX to encapsulate and deliver SARSr-CoV glycoproteins, with and without adjuvants[116], e.g. Matrix M1 (Isconova, Sweden) which has been shown to significantly enhance the immune response in mice to SARS-CoV spike proteins[18]. For efficiency and to reduce costs, initial trials will be conducted in the USA with locally acquired insectivorous big brown bats (*Eptesicus fuscus*) which we have maintained and housed for several experiments at our facility previously[25,26]. We will treat bats via topical application with various test formulations that include the biomarker Rhodamine B (RB), co-house them with untreated bats, and monitor transfer between bats by collecting hair and whiskers for biomarker analysis.

Initial field trials: Bat are not attracted to baits, so delivery in the field is challenging. The high rates of self and mutual grooming observed in bats has previously been exploited to cull vampire bats using poisons like warfarin, applied topically to a small number of bats. Once released, contact and mutual grooming transfers the poison within the colony. We have conducted preliminary biomarker studies in vampire bats in both Mexico and Peru and also in insectivorous bats in Wisconsin. In Peru, we conducted trials with RB-labeled glycerin jelly. Based on capture-recapture data, we estimated a rate of transfer from 1.3 – 2.8 bats for every bat marked. We are analyzing factors associated with rates of transfer, e.g. sex and age of initially treated bats, time of day, to model the rate of vaccination and impact on rabies transmission with different rates of application, prior to actual deployment of vaccine in the field. More recently, we applied RB marked glycerin jelly to the entry of bat houses used by little brown bats (*Myotis lucifugus*). Of 29 bats trapped one week post-application, 59% were positive for biomarker indicating they had eaten the jelly. We will conduct initial trials with each of the delivery vehicles in caves in Wisconsin, targeting local US insectivorous bats. Within one week of application, bats will be trapped at the cave entrance using mist nets or Harp traps and hair will be collected to assess the rate of uptake via biomarker analysis. The bats will be released immediately afterward. The procedures will be tested at several different locations as it will likely take some manipulation to determine appropriate dosages for maximum uptake. After we have determined the most optimal approaches for mass delivery, we will then test them on wild bats in our three cave sites in Yunnan Province. Again, biomarker will be used to assess rates of uptake and this data can then be used in modeling studies to help determine the optimal rates of application of immunomodulating agents. Biomarker studies can also be used

to assess uptake by non-target species, an important consideration in evaluating safety. Fieldwork will be conducted in collaboration with Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance).

Prototype PARC FEA aerosolization platform: Once we have confirmed uptake in laboratory studies, we will then assess mass delivery methods in local caves and hibernacula (using biomarker-labeled mediums but without immunomodulatory substances). In collaboration with Dr. Jerome Unidad of Palo Alto Research Center (PARC), we will adapt their innovative aerosol technology to cave settings in the form of a field-deployable spray device triggered by timers and movement detectors at critical cave entry points. PARC's Filament Extension Atomization (FEA) (Fig. 15) can spray fluids with a wide-range of viscosities ranging from 1mPa-s to 100Pa-s using a roll-to-roll misting process (https://www.parc.com/services/focus-area/amds/). This will make it compatible with all the fluid formulations above including the immune-modulating formulations from TA2, gels and creams for topical delivery and Poxvirus formulations, making it a universal platform for inoculating bats. We will subcontract to PARC to develop a prototype FEA system for lab testing, optimize spray conditions for DEFUSE fluids, manipulate fluid formulation for targeted spreading and bioefficacy, and design a prototype field-deployable system. We will initially trial this on captive bats at NWHC, then on Wisconsin cave bats, then at our test sites in Yunnan Province, China. The field-deployable system will be motion-actuated, and on a timer so that bats will be targeted at fly-in and fly-out, and diurnal flying non-target species (e.g. cave swiftlets) avoided.

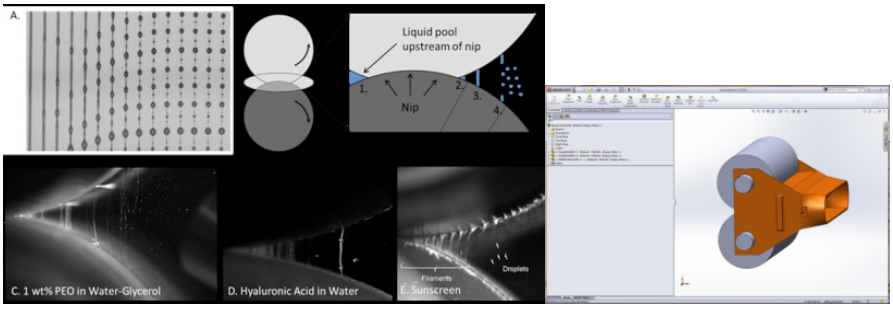

Fig. 15: Beads-on-a-string structures in a viscoelastic fluid in extension, B. Multiple beads-on-a-string formations in counter-rotating rollers (FEA), C. FEA spraying of PEO solution, D. hyaluronic acid, and E. sunscreen. Inset roller technology and portable sprayer design.

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Dynamic circulation modeling to optimize deployment strategy. To select amongst various options for immune boosting, priming, and targeting, and multiple delivery options and schedules, we will simulate deployment using a model of viral circulation in cave bat populations. The model will be fit to data from our three-cave test system but designed to be robust to be generalizable to other cases. We will simulate outcomes under a variety of different deployment scenarios to produce conservative estimate of necessary application under real-world conditions. *Fit stochastic viral circulation models to longitudinal sampling data:* We will use longitudinal viral prevalence, mark-recapture estimates of bat populations, radiotelemetry and infrared camera data collected during our field sampling to parameterize and construct models of bat population dynamics and viral circulation in our test caves. We will use a simple but robust stochastic SIR process model with immigration and emigration and flexible, nonlinear contact rates between bats[117]. This model structure can capture a wide range of viral dynamics from intermittent viral outbreaks to regular, endemic circulation with a relatively small number of parameters. We will fit these models to our sampling data using the **partially observable markov process (pomp)** framework[118], allowing estimates of the underlying latent dynamic disease transmission process, accounting for and separating the natural stochasticity of viral circulation and observation error in sampling. We will validate our models via temporal cross-validation: leaving out successive sections on longitudinal time-series from our model fitting to test the model, and by testing the results of a fit from two cave sites on data from a third. *Simulate circulation under a set of plausible deployment scenarios.* Using the top performing sets of immune boosting and targeted immune priming molecules from captive trials, and the delivery media and methods with the greatest uptake rates in cave studies, we will use the stochastic SIR model to generate simulations of viral circulation under a series of treatment deployments in our focal study caves. These scenarios will cover a range of plausible intensities, frequencies, and combinations of suppression strategies. They will incorporate uncertainty in the efficacy of each of the treatment strategies. From these simulations, we will estimate the expected degree and time period of suppression of viral circulation and shedding and the uncertainty in this expectations. We will determine the optimal scenario for deployment in our focal study caves. *Test robustness of deployment strategies under broader conditions:* We will use our simulation models to determine best strategies for deployment under a variety of conditions covering likely environments. We anticipate the deployment is likely to occur under (a) highly varied species population and compositions, with uncertain estimates based on rough observations (b) varied uptake and efficacy of immune boosting and targeting molecules due to different environmental conditions, and (c) limited time or resources to deploy treatment. Thus, we will simulate

deployment under many potential conditions to determine how optimal deployment differs according to condition, and determine deployment strategies which are conservative and robust to these uncertainties and limitations.

Proof-of-concept deployment of immune modulation molecules in test caves in Yunnan Province, China.

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MANAGEMENT PLAN

- Provide a summary of expertise of the team, including any subcontractors, and key personnel who will be doing the work. Resumes count against the page count.
- Identify a principal investigator for the project.
- Provide a clear description of the team's organization
- Include an organization chart with the following information, as applicable:
  A) Programmatic relationship of team members
  B) Unique capabilities of team members
  C) Task responsibilities of team members
  D) Teaming strategy among the team members
  E) Key personnel with amount of effort to be expended by each during each year
- Provide a detailed plan for coordination including explicit guidelines for interaction among collaborators/subcontractors of the proposed effort.
- Include risk management approaches.
- Describe any formal teaming agreements that are required to execute this program.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research organization focused on emerging zoonotic diseases. The PI, Dr. Peter Daszak, has 25+ years' experience managing lab, field and modeling research projects on emerging zoonoses. Dr. Daszak will commit 3 months annually to oversee and coordinate all project activities, and lead modeling and analytic work for TA1. Dr. Billy Karesh has 40+ years' experience leading zoonotic and wildlife disease projects, and will commit 1 month annually to manage

partnership activities and outreach. Dr. Jon Epstein, with 15 years' experience working emerging bat zoonoses will coordinate animal trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project. Support staff include field surveillance teams, modeling analysts, and consultants based in Yunnan Province, China, to oversee field trials. The EHA team has worked extensively with all other collaborators: Prof. Wang (15+ years); Dr. Shi (15+ years); Prof. Baric (5+ years) and Dr. Rocke (15+ years). **Subcontracts: #1** to Prof. Ralph Baric, UNC, to oversee reverse engineering of SARSr-CoVs, BSL-3 humanized mouse experimental infections, design and testing of immune priming treatments based on recombinant spike proteins. Assisted by senior personnel Dr. Tim Sheahan, Dr. Amy Sims, and support staff; **#2** to Prof. Linfa Wang, Duke NUS, to oversee the immune boosting approach, captive bat experiments, and analyze immunological and virological responses to immune boosting treatments; **#3** to Dr. Zhengli Shi, Wuhan Institute of Virology, to conduct PCR testing, viral discovery and isolation from bat samples collected in China, spike protein binding assays, and some humanized mouse work, as well as experimental trials on *Rhinolophus* bats. Her team will include Dr. Peng Zhou and support staff; **#4** to Dr. Tonie Rocke, USGS National Wildlife Health Center, to refine delivery mechanisms for both immune boosting and immune priming treatments. With a research technician, Dr. Rocke will use a captive colony of bats at NWHC for initial trials, and oversee cave experiments in China.

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based research organization focused on emerging zoonotic diseases. His >300 scientific papers include the first global map of EID hotspots[119,120], estimates of unknown viral diversity[121], predictive models of virus-host relationships[3], and evidence of the bat origin of SARS-CoV[29] and other emerging viruses [122-125]. He is Chair of the NASEM Forum on Microbial Threats, and is a member of the Executive Committee and the EHA institutional lead for the $130 million USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr. Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Dept. of Epidemiology and Dept. of Microbiology & Immunology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, cross species transmission and pathogenesis. His group has developed a platform strategy to access the potential "pre-epidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (REFS).

Prof. Linfa Wang is Director, Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore. His proven track record in the field includes identifying the bat origin of SARS-CoV, pioneering work on Henipaviruses and many more. His work has shifted from identifying the bat-origin of pathogens to understanding basic bat biology and the mechanisms by which they can endure sustained virus infection. He has received multiple awards including the 2014 Eureka Prize for Research in Infectious Diseases. He currently heads and administers a Singapore National Research Foundation grant on "Learning from bats" for $9.7M SGD. He is an advisory member of …. an Editor of multiple journals and current Editor-in-Chief for the Journal *Virology*.

Dr. Danielle Anderson is the Scientific Director of the Duke-NUS ABSL3 laboratory and is an expert in RNA virus replication. Dr Anderson has extensive experience in both molecular biology and animal models and will lead the animal studies. Dr Anderson has established Zika, Influenza and Reovirus non-human primate (NHP) models in Singapore, using different inoculation routes (such as mosquito inoculation), and has performed trials on over 30 NHPs.

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Dr Aaron Irving is an experienced postdoctoral fellow in the field of innate immunity and viral sensing with expertise focusing on host-pathogen interactions and intrinsic immunity. He oversees multiple projects on bat immune activation within Prof. Linfa Wang's laboratory at Duke-NUS Medical School and has experience in *in vivo* animal infection models.

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Prof. Zhengli Shi: Dr. Shi is the director of the Center for Emerging Infectious Diseases of the Wuhan Institute of Virology, Chinese Academy of Sciences. She got Ph.D training in Virology in Montpellier University II from 1996 to 2000, biosafety training at Australian Animal Health Laboratory in May 2006 and at Lyon P4 in October 2006. She is now in charge of the scientific activity in BSL3 and BSL4 of the Institute. Her research focuses on viral pathogen discovery through traditional and high-throughput sequencing techniques. She has been studying the wildlife-borne viral pathogens, particularly bat-borne viruses since 2004. Her group has discovered diverse novel viruses/virus antibodies in bats, included SARS-like coronaviruses, adenoviruses, adeno-associated viruses, circoviruses, paramyxoviruses and filoviruses in China. One of her great contributions is to uncover genetically diverse SARS-like coronaviruses in bats with her international collaborators and provide unequivocal evidence that bats are natural reservoir of SARS-CoV by isolation of one strain that is closely related to the SARS-CoV in 2002-3. She has coauthored >100 publications on viral pathogen identification, diagnosis and epidemiology.

Dr. Tonie Rocke is a

Dr. Peng Zhou is a
Dr. Xinglou Yang
Dr. Ben Hu

Dr. Kevin Olival is VP for Research at EcoHealth Alliance. His research over the last 15 years has focused on understanding the ecology and evolution of emerging zoonoses, with a focus on developing analytical tools and modeling approaches to forecast and prioritize the discovery and surveillance of viral zoonoses. This includes a recent large scale analysis identifying host and viral predictors of spillover in mammals [REF, Nature]. He has led several international field teams to investigate bat-borne viruses globally. Dr. Olival is the Modeling and Analytics coordinator for the USAID PREDICT-2 project; co-PI on an NIH-NIAID project to investigate CoVs in China; and PI on recent DTRA-CBEP grant to characterize CoVs from bats in Western Asia.

Please follow the same format and create Bios for all other personnel with Ph.D and higher. Peter Daszak will then work out how much space we have and decide who to include…

CAPABILITIES

- Describe organizational experience in relevant subject area(s), existing intellectual property, specialized facilities, and any Government-furnished materials or information.

- Discuss any work in closely related research areas and previous accomplishments.

(The following information was taken from the 'Goals and Impact' section of the abstract we submitted).

The SARSr-CoV-bat system, and immune modulation focus: Our group's 15 yrs work on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014 & WIV-1). We have shown they bind and replicate efficiently in primary human lung airway cells and that chimeras with SARSr-CoV spike proteins in a SARS-CoV backbone cause SARS-like illness in humanized mice, with clinical signs that are not reduced by SARS monoclonal therapy or vaccination. We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV *(7,8,9)*. We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**. Our work on bat immunology suggests that bats' unique flying ability has led to downregulated innate immune genes, and their ability to coexist with viruses such as SARSr-CoVs, henipa- and filoviruses that are lethal in many other mammals *(3)*. We have

identified bat-specific constitutively expressed bat interferon, a dampened STING-interferon production pathway (*4*, *5*), and have identified a series of other innate immunity factors that are dampened in bats (*6*).

STATEMENT OF WORK

- Provide a detailed task breakdown, citing specific tasks and their connection to the interim milestones and program metrics.

- Each phase of the program (Phase I base and Phase II option) should be separately defined in the SOW and each task should be identified by TA (1 or 2).

NOTE: The SOW must not include proprietary information.

- For each task/subtask, provide:

    - A detailed description of the approach to be taken to accomplish each defined task/subtask.

    - Identification of the primary organization responsible for task execution (prime contractor, subcontractor(s), consultant(s), by name).

    - A measurable milestone, i.e., a deliverable, demonstration, or other event/activity that marks task completion. Include quantitative metrics.

    - A definition of all deliverables (e.g. data, reports, software) to be provided to the Government in support of the proposed tasks/subtasks.

Phase I:

TA-01 Task 1.1 Construct species distribution models to predict viral spillover risk in cave bats in South and Southeast Asia
Sub-task 1.1.1.;lkj;lkj;klj
Sub-task 1.1.2.;lj;lkj;lkj
Deliverables: models capable of …..

TA-01 Task 2.5: Field studies to collect tolerant reservoir species. (EcoHealth Alliance, William Karesh).

Commented [PD41]: Below is formatted like the THUNDER proposal – need to follow that approach, with the example below of the sort of length

Sub-Task 2.5.1. Apply for and obtain IACUC approval and appropriate wildlife permits in Bangladesh for sample collection. Collection of blood and urogenital, oropharyngeal and rectal swab specimens from targeted bat, rodent and non-human primate species from Bangladesh (n = 1000 specimens). Collection of wing-punch dermal tissue biopsies from bats (n = 300).

Sub-Task 2.5.2. Field work is to be conducted by a trained field team using ethical, nondestructive capture, restraint, and sample collection techniques (with IACUC and local government approval). Samples are to be preserved in RNA later (or other preservative) to maintain cellular integrity and frozen at the point of collection using a liquid nitrogen dry shipper and maintained in -80^{o}C. All samples are to be shipped with appropriate government permission and export permits.

Deliverables: 1000 field specimens (whole blood, nasal/rectal swabs) collected from reservoir bats, rodents and non-human primates which have been obtained with all proper permits and permissions are appropriately shipped for further analysis.

TA1:

Task 1.1

Sub-task 1.1.1. Models to predict bat community in caves across S. and SE Asia.

Organization leading task: EcoHealth Alliance

Sub-task 1.1.2. Models to predict presence of viruses with zoonotic potential in bats across S. and SE Asia.

Progress Metrics:

- Joint species distribution model fit for Asian Bats
- Cave-level predictions of bat community composition
- Linear predictions of viral diversity in cave populations
- JSDM predictions of viral diversity in cave populations
- Prediction validations

Deliverable(s):

- Deployable spatial model software of bat community composition
- Deployable spatial model software of viral diversity in bat cave populations

Progress Metrics:

- Joint species distribution model fit for Asian Bats
- Cave-level predictions of bat community composition
- Linear predictions of viral diversity in cave populations
- JSDM predictions of viral diversity in cave populations
- Prediction validations

Deliverable(s):

- Deployable spatial model software of bat community composition
- Deployable spatial model software of viral diversity in bat cave populations

Subtask 1.1.3: Develop prototype app for the warfighter
Description and execution:
Preliminary Data:
Organization leading task: EcoHealth Alliance
Progress Metrics: Development of fully functional and user-friendly application. Use of application in the field.
Deliverables:

Task 1.2: Determining baseline risk of SARSr-CoV emergence in Yunnan, China
Subtask 1.2.1. Longitudinal sampling of bats to determine virus prevalence and diversity in Yunnan cave sites.
Subtask 1.2.2. Analyzing ability of CoVs to infect and emerge in people

***(TA1)* Subtask 5: Assay SARr-CoV quasispecies for spillover potential via assays for binding, cell entry, and pathogenesis in mouse models.**

Organization leading task: University of North Carolina

Progress Metrics: Not sure how to do this.

Deliverable(s):

1. *Methods to Produce Synthetic SARSr-CoV Virus Molecular Clones and Reverse Genetics.*
    a. ***Preliminary Data**:* Molecular Clones for SARSr-CoV WIV1, WIV16, SHC014 and HKU3-SRBD exist. We have demonstrated in the preliminary data that these reagents are already available.
    b. ***Target Goals**:* We will generate molecular constructs for 20+ chimeric SARSr-CoV encoding different S glycoprotein genes/yr
    c. **Target Goals***: We will generate 2-5 full length molecular clones of SARSr-CoV.*

2. *Methods of Recombinant virus Recovery and Characterization*
    a. **Preliminary Data***: Demonstrated recovery recombinant chimeric SARSr-CoV WIV1, WIV16, SHC014, HKU3-SRBD, including full length recombinant viruses of*

WIV1, WIV16, SHC014 and HKU3-SRBD.

b. *Target Goals:* We will isolate 20+ chimeric SARSr-CoV encoding novel S glycoprotein genes
c. *Target Goals:* We will isolate 2-5 full length SARSr-CoV/year/
    i. **Key Deliverables for Program-wide Success***: These two key reagents position us for immediate testing of the antiviral effects of* **broadscale immune boosting molecules** +/- immunogens on virus growth *in vitro* and *in vivo*, and on virus levels in models of chronic SARS-CoV infection in mice.

3. *Virus Phenotyping: Receptor Interactions and In Vitro Growth.*
    a. ***Preliminary Data****: Cell lines encoding bat, human, civet and mouse ACE2 receptors exist and have been validated. We have demonstrated the use of primary human airway epithelial cultures to characterize SARSr-CoV pre-epidemic potential.*
    b. ***Target Goals****: We will characterize SARSr-CoV recombinant virus growth in Vero cells, nonpermissive cells encoding the civet, bat and human ACE2 receptors.*

4. *Virus Pathogenic Potential in Humans:*
    a. *Preliminary Data: We also have transgenic human ACE2 mouse models to compare the pathogenic potential of SARSr-CoV*
    b. *Target Goals: We will evaluate SARSr-CoV pathogenic outcomes in hACE2 transgenic mice.*

5. *Virus Antigenic Variation:*
    a. *Preliminary Data: We have robust panels of broadly cross reactive human monoclonal antibodies against SARS and related viruses and mouse models to evaluate protection against SARSr-CoV replication and pathogenesis.*
    b. *We will evaluate SARS-vaccine performance against a select subset of SARSr-CoV (10), chosen based on the overall percent of antigenic variation, coupled with distribution across the S glycoprotein structure.*

6. *Low Abundant High Consequence Sequence Variants:*
    a. *We will identify the presence of low abundant, high risk SARSr-CoV, based on deep sequencing data*

7. *Proteolytic Processing and Pre-epidemic Potential.*
    a. *We will evaluate the role of proteolytic cleavage site variation on SARSr-CoV*

cross species transmission and pathogenesis in vivo.

(TA1) **Subtask 4: Build models to predict viral species spillover potential and evoluation**

Organization leading task: EcoHealth Alliance

Description and execution:

Progress Metrics:

- *Development of prior-based pathogenicity predictions and sequence testing guidance*
- *Model fits from initial rounds of viral characterization*
- *Model fits from secondary rounds of viral characterization*
- *Predictions of spillover probability of sequenced viral QS*
- *Deployable predictive model*

Deliverable(s):

- Fit models as reproducible, deployable software providing virus spillover potential predictions and uncertainties based on input of host species and viral sequence data
- Ranking of potential pathogenicity of virus QS from both Task X sampling and previous data.

(TA2) **Task 5: Trial experimental approaches aimed towards 'Broadscale Immune Boosting' using experimental bat colonies**

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

Organization leading task: Wuhan Institute of Virology, Duke-NUS

(TA2) **Task 6: Trial experimental approaches aimed towards 'Immune Targeting' using experimental bat colonies**

Organization leading task: University of North Carolina

Progress Metrics:

Deliverable(s):

1. ***Chimeric S-Glycoprotein Antigen Design, Recovery and Phenotyping for Immune Boosting***.

a. **Preliminary Data***: Demonstrated recovery recombinant chimeric HKU3-S_{mix}, demonstrating preservation of entry functions in the chimeric spike. Neutralizing epitopes and in vivo pathogenesis phenotypes were also preserved. Chimeric Spikes are biologically functional.*
b. ***Target Goals****:* We will isolate chimeric HKU3-S_{S014} S and WIV-S_{S014} genes, chimeric viruses and express the S glycoprotein from VRP and raccoon poxvirus expression vectors.
c. ***Target Goals:*** We will synthesize 2-3 chimeric S glycoproteins, recover recombinant viruses derived from natural recombinants in the population genetic structure of SARSr-CoV. We will also characterized recombinant protein expression from VRP and raccoon poxviruses.
d. ***Target Goals****:* We produce sufficient recombinant HKU3-S_{S014}, WIV-S_{S014} and HKU3-S_{mix} S glycoproteins for inclusion in nanoparticle and microparticle delivery vehicles.
    i. **Key Deliverables for Program-wide Success***: These two key reagents position us for immediate testing of the antiviral effects of* **broadscale immune boosting molecules** +/- immunogens.

2. ***Virus Phenotyping: Receptor Interactions and Growth in vitro and in vivo****.*
    a. ***Preliminary Data****: We have well developed metrics for evaluating chimeric S glycoprotein function in the context of whole virus, neutralization phenotypes and expression as recombinant proteins vaccines for testing in mice.*
    b. ***Target Goals****: Demonstrate chimeric S function in the context of virus infection in Vero and HAE cells and susceptibility to neutralizing antibodies targeted the SARS RBD.*
    c. ***Target Goals****: Evaluate chimeric virus pathogenesis in hACE2 transgenic mice and the ability of VRP vaccines encoding chimeric spikes to elicit protective immunity against lethal SARS-CoV, HKU3-S_{mix} and SCH014 challenge.*

3. ***Production of Agonist (TLR4, dsRNA, Sting) and Chimeric S glycoprotein Nanoparticle and Microparticle Suspensions for in vivo studies***
    a. ***Preliminary Data****: Robust preliminary data exists on the production and immunogenicity of nanoparticle and microparticle delivery systems.*
    b. ***Target Goals****: Produce nanoparticle and microparticle delivery systems encoding agonists, coupled with in vitro testing in vitro in bat and in other reporter cells, mice and bats.*
    c. ***Target Goals****: Inclusion of chimeric recombinant proteins and agonists in nanoparticle and microparticle delivery vehicles, coupled with testing in vitro and*

in vivo in mice and bats.

d. ***Target Goals***: Perform *in vivo* testing in collaboration with Dr. Shi and Dr. Wang.

SCHEDULE AND MILESTONES

- Provide a detailed schedule showing tasks (task name, duration, work breakdown structure element as applicable, performing organization), milestones, and the interrelationships among tasks.

  NOTE: Task structure must be consistent with that in the SOW.

- Measurable milestones should be clearly articulated and defined in time relative to the start of the project.

PREEMPT TRANSITION PLAN

- Indicate the types of partners (e.g. government, private industry, non-profit)
- Submit a timeline with incremental milestones toward successful engagement. NOTE: begin transition activities during the early stages of the program (Phase I).
- Describe any potential DARPA roles.

PREEMPT RISK MITIGATION PLAN

- Provide the following:
  - An assessment of potential risks to public health, agriculture, plants, animals, the environment, and national security.
  - Guidelines the proposer will follow to ensure maximal biosafety and biosecurity.
  - A communication plan that addresses content, timing, and the extent of distribution of potentially sensitive dual-use information. The plan must also address how input from DARPA, other government, and community stakeholders will be taken into account in decisions regarding communication and publication of potentially sensitive dual-use information.

Commented [PD42]: Description from the BAA:

PREEMPT Transition Plan
Proposers must include a PREEMPT Technology Transition Plan. Proposers must indicate the types of partners (e.g. government, private industry, non-profit) they plan to pursue and submit a timeline with incremental milestones toward successful engagement. Proposers should begin transition activities during the early stages of the program (Phase I). Awardees must include
DARPA in the development of transition relationships. If the transition plan includes a start-up company, a business development strategy must be included as well. The extent by which the proposed intellectual property (IP) rights will impede the Government's ability to transition the technology will be considered in the proposal evaluation.

ETHICAL, LEGAL, SOCIETAL IMPLICATIONS

- Address potential ethical, legal, and societal implications of the proposed technology.

BIBLIOGRAPHY

A) Brief Bibliography (no page limit indicated – can be published/unpublished)
This and next part don't count toward 36 page limit

RELEVANT PAPERS

B) Up to 3 relevant papers attached (optional)
Propose:

- Ge et al. Nature
- Menacherry et al.
- Zhou et al. SADS-CoV

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Please reply to this email - DARPA DEFUSE draft v2 - Technical Plan

Peter Daszak <daszak@ecohealthalliance.org>

Thu 3/22/2018 12:56 AM

To: Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; Rocke, Tonie E <trocke@usgs.gov>; Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>
Cc: Danielle Anderson (danielle.anderson@duke-nus.edu.sg) <danielle.anderson@duke-nus.edu.sg>; aaron.irving@duke-nus.edu.sg <aaron.irving@duke-nus.edu.sg>; Baric, Toni C <antoinette_baric@med.unc.edu>; sims0018@email.unc.edu <sims0018@email.unc.edu>; Luke Hamel <hamel@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>

All – please don't reply to the last email, I had Toni Baric's wrong email address – corrected now!

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Peter Daszak
Sent: Thursday, March 22, 2018 1:53 AM
To: Zhengli Shi (zlshi@wh.iov.cn); Ralph Baric (rbaric@email.unc.edu); 周鹏 (peng.zhou@wh.iov.cn); 'Wang Linfa'; Rocke, Tonie; Jerome.Unidad@parc.com
Cc: Danielle Anderson (danielle.anderson@duke-nus.edu.sg); 'aaron.irving@duke-nus.edu.sg'; 'antonette_baric@med.unc.edu'; 'sims0018@email.unc.edu'; Luke Hamel (hamel@ecohealthalliance.org); Noam Ross; Kevin Olival, PhD (olival@ecohealthalliance.org); Jon Epstein; William B. Karesh; Hongying Li; 'Anna Willoughby (willoughby@ecohealthalliance.org)'
Subject: DARPA DEFUSE draft v2 - Technical Comment
Importance: High

Dear all,

Apologies for the delay - here's the draft Technical Plan for our proposal with everyone's section incorporated, edited and shortened.

Please ignore all other sections – these are being worked on by others. We're only editing the Technical Plan right now.

Can each of you go through your respective section and, with one of you acting as the point person to coordinate edits and responses from your teams:

1. Answer any questions in comment boxes

2. Insert any missing references – please just cut and paste the ref as a word doc into a comment box rather than inserting the endnote reference at this point

3. Read through your sections and suggest edits. Best if you use lots of comment boxes, but also OK if you start editing using 'track changes'. NB – we need to reduce the length probably by one third, so any suggestions and cuts would be most appreciated! Also, please just keep this as a Word doc for now – there are formatting issues when converting backwards and forwards into Google docs and Word.

4. Ralph and team – please provide higher res images for all those in this draft, and please make sure they're editable – i.e. we can take out the text and alter each icon within each image

5. All – please check the language I've used and correct any glaring errors.

6. Can you get edits back to me, cc'd to Luke and Anna by **Saturday 9am Eastern (New York) time,** at which point I'll start trimming it back into the page limit and incorporating all the other sections.

While you're working on these sections, I'll be editing the rest of the proposal with Luke, Anna and others.

Cheers,

Peter

Peter Daszak
President

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

-----Original Message-----
From: Peter Daszak
Sent: Tuesday, February 27, 2018 2:14 PM
To: Zhengli Shi (zlshi@wh.iov.cn); Ralph Baric (rbaric@email.unc.edu); 周鹏 (peng.zhou@wh.iov.cn); 'Wang Linfa'; Rocke, Tonie
Cc: Danielle Anderson (danielle.anderson@duke-nus.edu.sg); 'aaron.irving@duke-nus.edu.sg'; 'antonette_baric@med.unc.edu'; 'sims0018@email.unc.edu'; Luke Hamel (hamel@ecohealthalliance.org)
Subject: For our DARPA PREEMPT conversations this week: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status
Importance: High

Dear All,

Good news from DARPA - they like our abstract and we're officially invited for a full proposal. From the attached letter, it looks like they've got a lot of proposals asking for too much $$$, but there are some clear ways we can hedge against any possible cuts. We can talk further about this, and about fleshing out the technical details on our calls this week.

I'm working on scheduling a call with the DARPA team for Thursday of Friday this week - 15 mins to go through how these bullets in the letter above will affect our full proposal. It'll just be me and Luke, but we can think about key questions to ask them..

Re. the full proposal. Luke has taken the abstract text and started populating the full proposal framework (attached), to give us an idea of what we need to write. It's not a huge effort, but it'll have to be technically sound, but still tell the overall 'story' that DARPA want to hear - i.e. we can provide proof-of-concept of blocking spillover based on this novel and interesting approach.

Look forward to talking with all of you.

Cheers,

Peter

Peter Daszak
President

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-----Original Message-----
From: PREEMPT [mailto:PREEMPT@darpa.mil]

Sent: Tuesday, February 27, 2018 8:51 AM
To: (b) (6)
Cc: (b) (6)
Subject: HR001118S0017-PREEMPT-PA-001 Proposal Abstract Status

(b) (6)

Thank you for your interest in the Biological Technologies Office's PREventing EMerging Pathogenic Threats (PREEMPT) program. Please find your proposal abstract status attached.

Regards,

BAA Coordinator
Contractor Support to DARPA/BTO
PREEMPT@darpa.mil

[EXTERNAL] PREEMPT - A few important items

Luke Hamel <hamel@ecohealthalliance.org>

Fri 3/23/2018 1:45 PM

To: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>
Cc: Rocke, Tonie E <trocke@usgs.gov>; Jonathon Musser <musser@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>

Hi Rachel and Katherine,

I wanted to address a few important PREEMPT items with you:

- Regarding **NWHC budget justification**
  - If you have not already done so (and I apologize for not knowing the answer), could you please send us your budget justification document? We are hoping to have all collaborator budgets and budget justifications as soon as possible.
- Regarding language on **'Long-term safety and efficacy'**
  - In the PREEMPT proposal, we must state how we will establish methods to assess the 'long-term safety and efficacy of our preemptive approaches'
    - Given your field of work, do you have any existing language on how to address potential negative impacts of intervention approaches on non-target species?
    - I have attached language from the BAA to provide you with further guidance on what DARPA requires us to include.
    - This being said - Rachel and Katherine, could you please write-up a short section (a paragraph or so), that addresses this issue 'long-term safety and efficacy'?
      - I apologize for the extremely short notice, but **we would greatly appreciate it if you could return this to us by tomorrow afternoon, Sat. (3/24).**
- Regarding '**pricing assumptions'** for NWHC facilities
  - Previously, we had asked you to identify any 'pricing assumptions' that may correspond with use of government facilities. Due to confusion about what exactly was being asked for, we reached out to DARPA staff, asking them to clarify the matter:
    - We asked: "EcoHealth Alliance has a USG entity listed as a subcontractor in our proposal. Is the USG entity required to identify any pricing assumptions beyond those within their fully detailed and documented budget?
    - To which they responded: "No"
      - Long story short...there is NO need for you to identify any additional pricing assumptions.

Thank you and please let me know if you have any questions. I will be available by email and phone (mobile number listed below) over the weekend, should you need to contact me.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

J. **PREEMPT Risk Mitigation Plan** (see Section 1.4): Proposers must provide a risk mitigation plan that addresses the following:

1) An assessment of potential risks to public health, agriculture, plants, animals, the environment, and national security.
2) Proposed guidelines that the proposer will follow to ensure maximal biosafety and biosecurity during the course of the research.
3) A communication plan that addresses content, timing, and the extent of distribution of potentially sensitive dual-use information. The plan must also address how input from DARPA, other government, and community stakeholders will be taken into account in decisions regarding communication and publication of potentially sensitive dual-use information.

TA2 Components:

Technical Area 2 aims to develop deployable and scalable methods to preempt viral jump to other species. Proposers must address, at minimum, all of the following aspects:

1) Proof-of-concept preemption approaches;
2) Scalable delivery methods;
3) **Analysis of long-term sustainability; and**
4) Experimental validation.

3. Analysis of long-term safety and efficacy

Proposers must establish initial methods to assess the long-term safety and efficacy of preemptive approaches (e.g., determine the mechanism by which species specificity of a vaccine is maintained, and assess evolutionary stability and ecological safety).

Re: [EXTERNAL] PREEMPT - A few important items

Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>

Fri 3/23/2018 2:19 PM

To: Luke Hamel <hamel@ecohealthalliance.org>
Cc: Richgels, Katherine L <krichgels@usgs.gov>; Rocke, Tonie E <trocke@usgs.gov>; Jonathon Musser <musser@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>

Hi Luke,
I will write the paragraph on long-term safety and efficacy and send it to you later this afternoon (I'll be on vacation for a few days starting tomorrow).
I don't know what the budget justification document is and only have budget files that Tonie has already sent to you. I have attached them here. If it is a specific form we need to fill out, Katie might be the best person to enter the required information.
--Rachel

On Fri, Mar 23, 2018 at 1:45 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Hi Rachel and Katherine,
>
> I wanted to address a few important PREEMPT items with you:
>
> - Regarding **NWHC budget justification**
>   - If you have not already done so (and I apologize for not knowing the answer), could you please send us your budget justification document? We are hoping to have all collaborator budgets and budget justifications as soon as possible.
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>   - We asked: "EcoHealth Alliance has a USG entity listed as a subcontractor in our proposal. Is the USG entity required to identify any pricing assumptions beyond those within their fully detailed and documented budget?
>   - To which they responded: "No"
>   - Long story short...there is NO need for you to identify any additional pricing assumptions.
>
> Thank you and please let me know if you have any questions. I will be available by email and phone (mobile number listed below) over the weekend, should you need to contact me.
>
> Best,

Luke Hamel
Program Assistant

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EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

--
Rachel Abbott
USGS National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711
(608) 270-2489
Fax: (608) 270-2415

Task 7: Develop and assess delivery methods to bats for immune boosting and priming molecules

Description and execution: While work is proceeding to identify and optimize immunomodulating agents to manage SARS-Coronaviruses, we will concurrently develop and test mediums, routes, and methods of delivery to large colonies of bats. Several different approaches or combinations of approaches will be assessed to determine the most feasible and simplest method of delivery that achieves high uptake by bats, is safe for humans as well as target and non-target species, and minimizes disturbance to the colony. Sticky edible gels or pastes that bats groom from themselves and each other have been used previously to deliver pharmaceuticals to bats orally and are currently being tested as a medium for delivery of vaccines against rabies and other diseases in wild bats (see preliminary data). These may also be useful for delivering immune modulators and recombinant SARSr-CoV spike proteins to *Rhinolophus* bats, but may need to be combined with viral vectors (like poxvirus or adenovirus) or nanoparticles/nanoemulsions that enhance uptake through mucous membranes or transdermally after topical application.

Poxviruses in particular have been demonstrated to be effective viral vectors for delivering vaccines to wildlife (Slate et al., 2009) Freuling et al., 2013; Rocke et al., 2017). Recent laboratory studies in bats have shown that poxviruses can replicate safely at high levels in bats after oronasal administration (Stading et al., 2016)m and poxvirus vectored vaccines are immunogenic, protecting bats from rabies challenge (Stading et al 2017; see preliminary data). Poxviruses are highly safe, having been tested in a wide variety of wild and domestic animals, they allow for large inserts of foreign DNA, and they have a proven record of success. Poxviruses are good candidates for this project, but we will also consider others.

In addition to viral vectors, we will also consider methods to achieve transcutaneous delivery of the immune boosting proteins without the use of live agents. Recent advances in methods to achieve transdermal or transcutaneous delivery of drugs and vaccines have been reported. (Roberts et al., 2017). However, a major impediment to this route of vaccination is the stratum corneum, the outermost barrier layer of the skin that protects underlying layers from infection and damage. Numerous approaches have relied on mechanical methods to compromise the stratum corneum to allow the drug or vaccine to penetrate into the skin (Roberts et al., 2017). Innovations in nanotechnology show promise in being able to deliver drugs and vaccines into the deeper layers of the skin without the need for damage to the stratum corneum (Mishra et al., 2013), an important consideration. Dendritic cells and Langerhans cells, antigen-presenting cells which reside in the dermis and epidermis, can take up these transdermally delivered proteins and generate an immune response. We are currently testing poly lactic-co-glycolic acid (PLGA) as a nanoparticle to encapsulate rabies glycoprotein as a method of transcutaneous delivery of vaccine to bats. PLGA has been used previously to deliver both toll-like receptor agonists and antigens simultaneously to mice (Ebrahimian, 2017). This and other products (outlined above in Task ?) could potentially be useful with SARSr-CoV glycoproteins. Adjuvants can also be incorporated into nanoemulsions and nanoparticles to amplify the natural immune response to the vaccine antigens (Karande and Mitragotri, 2010). With SARS-CoV spike proteins, the adjuvant Matrix M1 (Isconova, Sweden) has been shown to significantly enhance the immune response in mice (Coleman et al. 2014)

In collaboration with Dr. Baric and others, we will determine the most likely immunomodulating formulations based on the results of TA2, previous animal studies and other available data and then use both laboratory and field studies to assess and optimize delivery vehicles and methods for wild bats. To reduce costs, initial studies will be conducted with locally acquired insectivorous bats (*Eptesicus fuscus*--big brown bats). We have successfully maintained and housed big brown bats and other insectivorous species for several experiments at our facility previously (Stading et al., 2016, 2017). We will treat bats via topical application with various test formulations that include the biomarker Rhodamine B (RB), co-house them with untreated bats, and monitor transfer between bats by collecting hair and whiskers for biomarker analysis. Rhodamine B is detectable within the hair of animals within 24 hours of consumption using a fluorescence microscope, and we have considerable experience using this biomarker for similar studies (see preliminary data).

Once we have confirmed uptake in laboratory studies, we will then assess mass delivery methods in local caves and hibernacula (using biomarker-labeled mediums but without immunomodulatory substances). In collaboration with Dr. Jerome Unidad of Palo Alto Research Center, we will explore the use of innovative aerosol technology that could be used in cave settings in the form of a field-deployable spray device triggered by timers and movement detectors at critical cave entry points. The PARC technology called Filament Extension Atomization (FEA) can spray fluids with a wide-range of viscosities ranging from 1mPa-s to 100Pa-s using a roll-to-roll misting process (further details in the PARC website). This will make it compatible with all the fluid formulations mentioned earlier including the immunomodulating formulations from TA2, gels and creams for topical delivery and Poxvirus formulations, making it a universal platform for inoculating the bats. Within one week of application, bats will be trapped at the cave entrace using mist nets or Harp traps and hair will be collected to assess the rate of uptake via biomarker analysis. The bats will be released immediately afterward. The procedures will be tested at several different locations as it will likely take some manipulation to determine appropriate dosages for maximum uptake. After we have determined the most optimal approaches for mass delivery, we will then test them on wild bats in our three cave sites in Yunnan Province. Again, biomarker will be used to assess rates of uptake and this data can then be used in modeling studies to help determine the optimal rates of application of immunomodulating agents. Biomarker studies can also be used to assess uptake by non-target species, an important consideration in evaluating safety. Fieldwork will be conducted in collaboration with Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance).

Preliminary Data: Rocke and colleagues have developed oral vaccines and delivery methods to manage disease in free-ranging wildlife for many years, including a sylvatic plague vaccine for prairie dogs (Rocke et al., 2017), and more recently, vaccines against rabies (Stading et al., 2017) and white-nose syndrome for bats (Rocke, unpublished data). In addition to developing, testing and registering vaccines for experimental field use, vaccine delivery methods and uptake by the target species were optimized using biomarker studies prior to deployment; biomarker studies were also used to assess uptake and safety in non-target hosts (Tripp et al., 2015). A similar approach will be used to develop, test and optimize delivery methods to *Rhinolophus* bats in SE Asia.

To manage plague caused by *Yersinia pestis* in prairie dogs, a raccoon poxvirus vectored vaccine expressing plague antigens was incorporated into a peanut-butter flavored bait matrix. Rhodamine B (RB), a biomarker that dyes hair, whiskers and feces and is visible within 24 hours of consumption by animals, was included in the baits in order to assess uptake by both target and non-target species (Figure 1). When viewed under a UV microscope at a specific wavelength, the biomarker is visible until the hair grows out (approximately 50 days in prairie dogs). Biomarker studies were initially used to assess palatability and acceptance of the bait matrix by wild prairie dogs (Tripp et al., 2014) and also used to assess bait ingestion by non-target rodents (Tripp et al., 2015). After safety was confirmed in non-targets and with the approval of USDA Center for Veterinary Biologics, a large field trial was conducted over a 3-year period that demonstrated vaccine effectiveness in four species of prairie dogs in seven western states (Rocke et al., 2017). Using biomarker analysis, we then assessed site- and individual host-level factors related to bait consumption in prairie dogs to determine those most related to increased bait consumption, including age, weight, and the availability of green vegetation. Identifying the factors that maximize the likelihood of expedient bait uptake by targeted individuals is important for developing strategies to optimize vaccine effectiveness. This will also be important in developing disease management strategies for bats.

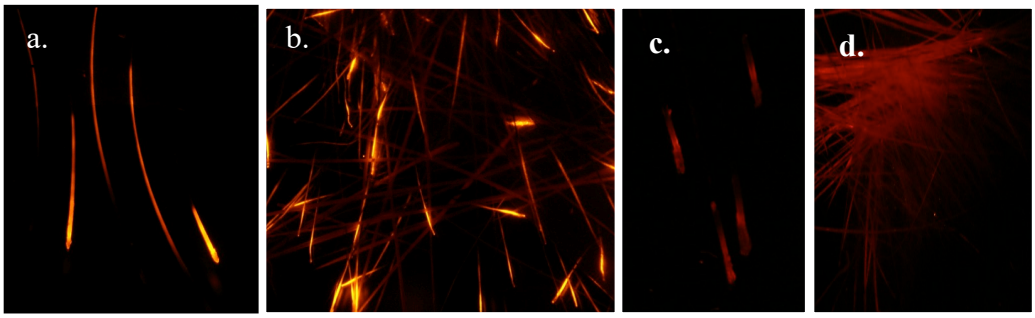

Figure 1. Prairie dog hair and whisker samples viewed under fluorescence microscope (excitation wavelength: 540 nm, emission wavelength: 625 nm) to determine uptake of baits containing Rhodamine B. a) whiskers positive for RB uptake 20 days after bait distribution, b) hair sample positive for RB uptake 16 days after bait distribution, c and d) whiskers and hair negative for RB uptake 20 days after bait distribution (note natural dull fluorescence).

In recent years, our research team has been developing and testing vaccines and delivery methods for use in free-ranging bats. First we tested two commonly used viral vectors, modified vaccinia Ankara (MVA) and raccoon poxvirus (RCN), for their safety and replication in bats using in vivo biophotonic imaging. (Stading et al. 2017). RCN replicated to higher levels in bats than MVA, even via the oral route, and was found to be highly safe for bats (Figure 2). We then used raccoon poxvirus as a viral vector to express a novel rabies glycoprotein (mosaic or MoG) and tested the protective efficacy of this construct in bats after both oronasal and topical administration (Stading et al 2017). Both methods of application were successful, protecting nearly all of the immunized and challenged bats (Figure 3), work is now progressing to develop methods of vaccine delivery to vampire bats, one of the primary reservoirs of rabies for both humans and

animals, primarily cattle, in several Latin American countries. We are also using a similar approach to develop vaccines for white-nose syndrome in bats, a devastating disease that has killed millions of insectivorous bats in North America.

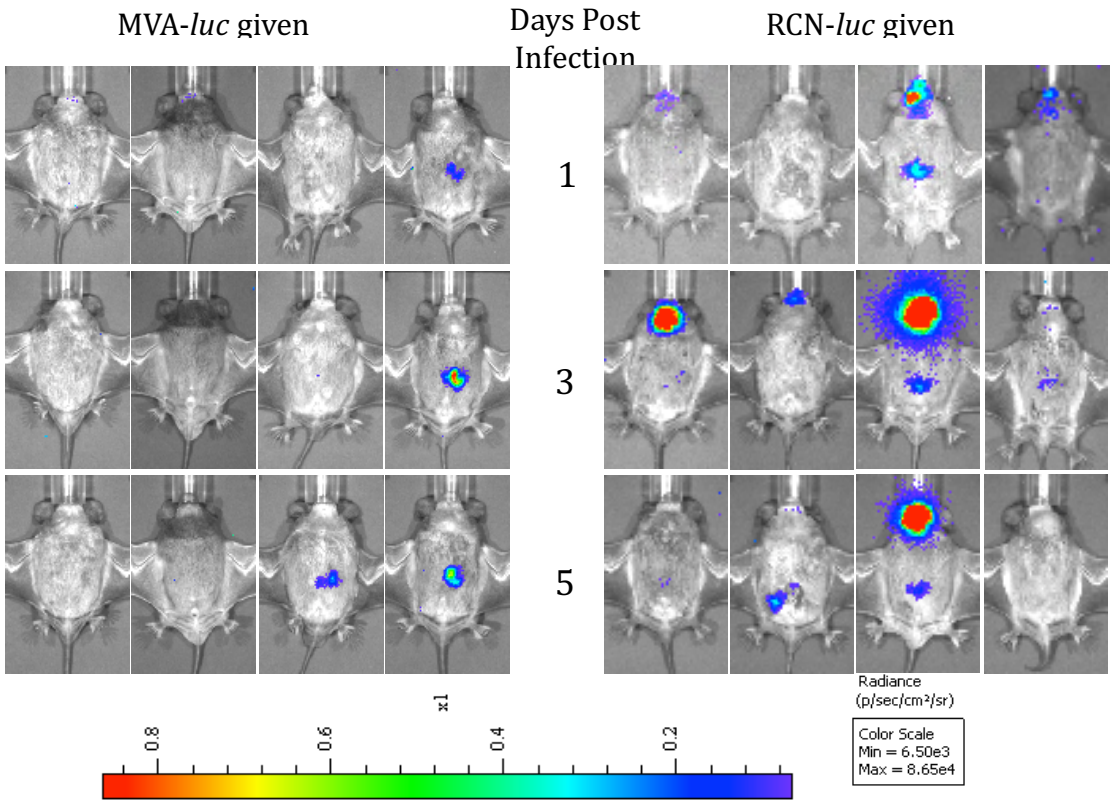

Figure 2. Luminescence, indicative of viral replication of modified vaccinia Ankara (MVA) and raccoon poxvirus RCN) in *Tadarida brasiliensis* on days 1, 3 and 5 post-inoculation via the oronasal route.

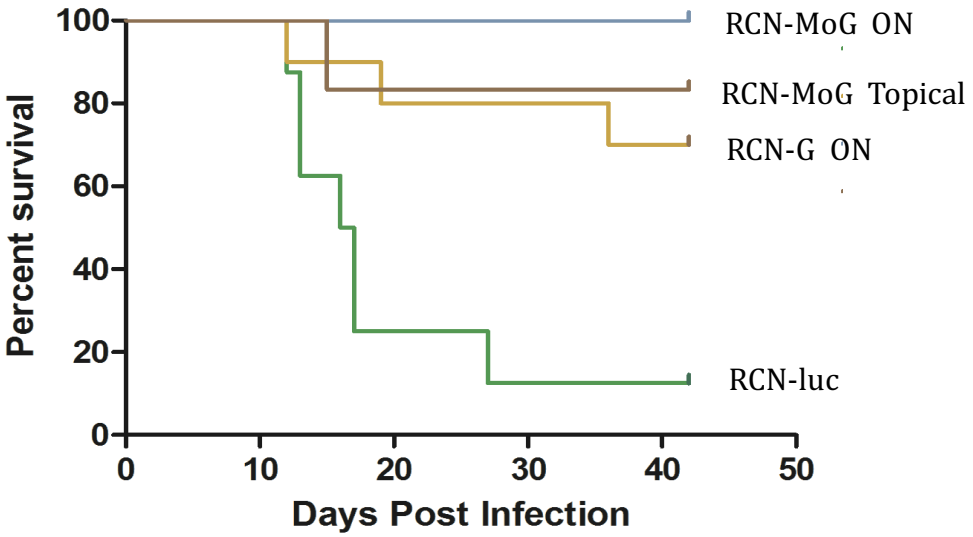

Figure 3. Results of vaccine efficacy and rabies challenge trials in *Epstesicus fuscus* immunized with raccoon poxvirus expressing a mosaic G protein (RCN-MoG) either oronasally (ON) or topically in comparison to RCN expressing typical G protein and RCN expressing luciferase (a negative control).

For bats a different approach is required for vaccine delivery, as in general, they are not attracted to baits. Bats, especially vampire bats, are known to practice self and mutual grooming at a high rate, and this behavior has been exploited to cull vampire bats using poisons like warfarin. The poison is applied topically to a number of bats that are released. When they return to their roost, the poison is transferred to roost-mates by contact and mutual grooming. We are exploiting this same behavior for vaccine application. Preliminary biomarker studies (without vaccine) are being conducted in vampire bats in both Mexico and Peru and also in insectivorous bats in Wisconsin. In a pilot study in Peru, we treated 50 bats from a single cave with RB-labelled glycerin jelly. Based on capture-recapture data, we estimated the population at ~200 bats, so ~25% of bats were initially marked. Upon trapping of this population a few days later, 64 bats were captured, including 19 originally marked bats (Table 1 – could be made into a figure instead). Hair was collected and examined for RB marking under a fluorescence microscope. All treated bats were positive for RB marking in addition to 39% of newly captured bats, indicating a rate of transfer of about 1.3 bats for every bat marked. Additional trials have been conducted, with transfer rates of up to 2.8 bats for every bat treated achieved at least once. These trials are being analyzed to assess factors associated with rates of transfer, e.g. sex and age of initially treated bats, time of day, etc. This data is then being used to model the rate of vaccination and impact on rabies transmission with different rates of application, prior to actual deployment of vaccine in the field.

Table 1. Marking of vampire bats a few days after application of glycerin jelly containing Rhodamine B.

	Number captured	Positive	Negative	Inconclusive	% positive (w/o inc)
All bats	64	34	25	5	58
Recaptured marked bats	19	18	0	1	100
New bat captures	45	16	25	4	39

For insectivorous bats, we are trying other approaches. Instead of hand applying the jelly to bats, we applied RB marked glycerin jelly to the entry of bat houses used by little brown bats (*Myotis lucifugus*). The bats became covered as they entered the houses and then consumed the material during self and mutual grooming. One week later, bats were trapped at the houses to determine the rate of uptake. Of 29 bats trapped one week post-application, 59% (17) were positive for biomarker indicating they had eaten the jelly. Thus, with additional optimization, application of vaccine to bat houses or other structures (small cave entrances) could also be a viable method of delivery. In addition, we are considering different spray applications directly to roosting bats in caves and through motion-sensing sprayers at cave entrances. Whatever the means of application, effective treatment relies on ingestion by bats, and that is easily confirmed with the use of the biomarker, RB.

PARC will develop the FEA aerosol technology wide-scale inoculation of bats in PRE-EMPT. Fig.4 shows the basic principle of the technology and the resulting spray from representative fluids (aqueous polymer solutions, consumer formulations). FEA technology can be used for the full range of fluids of interest to the program including gels and creams for topical application and aqueous/non-aqueous vaccine formulations. Further details can be found in the PARC website (see references).

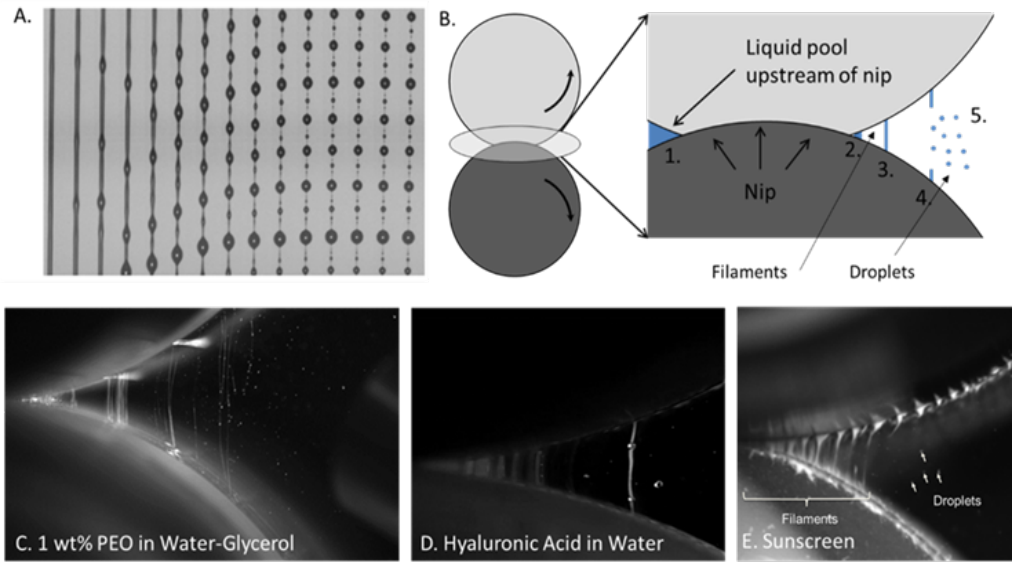

Figure 4. FEA technology: A. Beads-on-a-string formation in viscoelastic fluids in extension (Oliveira and McKinley, 2005), B. Roll-to-roll parallelization of filament formation and break-up in FEA, C.-E. Examples of fluids sprayed with FEA including polyethylene oxide in water-glycerol (C.), hyaluronic acid in water (D.) and sunscreen (E.)

Organization leading task: USGS National Wildlife Health Center
Participating organizations: Palo Alto Research Center (PARC)

Progress Metrics: Not sure exactly what format to use here

Deliverable(s):
Medium and methods to deliver immunomodulatory agents to bats.
Data on uptake in insectivorous bats.
Reports, manuscripts, presentations.

Coleman CM, Liu YV, Mu H, Taylor JK, Massare M, Flyer DC, Smith GE, Frieman MB. 2014. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine 32:3169-3174.

Ebrahimian M, Hashemi M, Maleki M, Hashemitabar G, Abnous K, Ramezani M, Haghparast A. 2017. Co-delivery of dual toll-like receptor agaonists and antigen in poly(lactic-co-glycolic) acid/polyethylenimine cationic hybrid nanoparticles promote efficient in vivo immune responses. Front Immunol 8:1077.

Freuling CM, Hampson K, Selhorst T, Schro¨der R, Meslin FX, Mettenleiter TC, Mu¨ller

T (2013) The elimination of fox rabies from Europe: determinants of success and lessons for the future. Philosophical Transactions of the Royal Society London B Biological Sciences 368(1623):20120142 (DOI: 10.1098/rstb.2012. 0142)

Karande P, Mitragotri S. 2010. Transcutaneous immunization: an overview of advantages, disease targets, vaccines, and delivery technologies. Annu Rev Chem Biomol Eng 1:175-201.

Mishra DK, Dhote V, Mishra PK. 2013. Transdermal immunization: biological framework and translational perspectives. Expert Opin Drug Deliv 10:183-200.

Oliveira MSN, McKinley GH. 2005. Iterated stretching and multiple beads-on-a-string phenomena in dilute solutions of highly extensible flexible polymers. Physics of Fluids 17: 071704.

PARC Website: Advanced Manufacturing and Deposition Systems Group – https://www.parc.com/services/focus-area/amds/

Roberts MS, Mohammed Y, Pastore MN, Namjoshi S, Yousef S, Alinaghi A, Haridass IN, Abd E, Leite-Silva VR, Benson HAE, Grice JE. 2017. Topical and cutaneous delivery using nanosystems. J Control Release 247:86-105.

Rocke TE, Tripp DW, Russell RE, Abbott RC, Richgels KLD, Matchett MR, Biggins DE, Griebel R, Schroeder G, Grassel SM, Pipkin DR, Cordova J, Kavalunas A, Maxfield B, Boulerice J, Miller MW. 2017. Sylvatic plague vaccine partially protects prairie dogs (*Cynomys* spp.) in field trials. EcoHealth DOI: 10.1007/s10393-017-1253-x.

Slate D, Algeo TP, Nelson KM, Chipman RB, Donovan D, Blanton JD, Niezgoda M, Rupprecht CE (2009) Oral rabies vaccination in North America: opportunities, complexities, and challenges. PLoS Neglected Tropical Diseases 22 3(12):e549.doi:10.1371/journal.pntd.0000549

Stading BR, Osorio JE, Velasco-Villa A, Smotherman M, Kingstad-Bakke B, Rocke TE. Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). Vaccine. 2016;34: 5352–5358. doi:10.1016/j.vaccine.2016.08.088

Stading B, Ellison JA, Carson WC, Panayampalli SS, Rocke TE, Osorio JE. Protection of bats (*Eptesicus fuscus*) against rabies following topical or oronasal exporue to a recombinant raccoon poxvirus vaccine. PLoS Negl Trop Dis 11:e0005958.

Tripp DW, Rocke TE, Streich SP, Brown NL, Fernandez JR-R, Miller MW. 2014. Season and application rates affect vaccine bait consumption by prairie dogs in Colorado and Utah, USA. J Wildlife Dis 20:

Tripp DW, Rocke TE, Streich SP, Abbott RC, Osorio JE, Miller MW. 2015. Apparent field safety of a raccoon poxvirus-vectored plague vaccine in free-ranging prairie dogs, Colorado, USA. J Wildlife Dis 51:

WORKSPACE FORM

1-800-518-4726
SUPPORT@GRANTS.GOV

This Workspace form is one of the forms you need to complete prior to submitting your Application Package. This form can be completed in its entirety offline using Adobe Reader. You can save your form by clicking the "Save" button and see any errors by clicking the “Check For Errors” button. In-progress and completed forms can be uploaded at any time to Grants.gov using the Workspace feature.

When you open a form, required fields are highlighted in yellow with a red border. Optional fields and completed fields are displayed in white. If you enter invalid or incomplete information in a field, you will receive an error message. Additional instructions and FAQs about the Application Package can be found in the Grants.gov Applicants tab.

OPPORTUNITY & PACKAGE DETAILS:

Opportunity Number:	HR001118S0017
Opportunity Title:	PREventing EMerging Pathogenic Threats
Opportunity Package ID:	PKG00237724
CFDA Number:	12.910
CFDA Description:	Research and Technology Development
Competition ID:	
Competition Title:	
Opening Date:	01/19/2018
Closing Date:	03/27/2018
Agency:	DARPA - Biological Technologies Office
Contact Information:	BAA Coordinator PREEMPT@darpa.mil

APPLICANT & WORKSPACE DETAILS:

Workspace ID:	WS00094394
Application Filing Name:	Project DEFUSE
DUNS:	0770900660000
Organization:	ECOHEALTH ALLIANCE INC.
Form Name:	R & R Subaward Budget 10 YR Subform
Form Version:	1.4
Subform Name:	USGS Ntl. Wildlife Health Cen
Requirement:	Optional
Download Date/Time:	Mar 06, 2018 05:28:38 PM EST
Form State:	Error(s)

FORM ACTIONS:

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 1** **Start Date:** 12/01/2018 **End Date:** 11/30/2019

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.85			9,179.00	2,475.00	11,654.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 88,630.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00	0.00	24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel 24,782.00

Total Salary, Wages and Fringe Benefits (A+B) 113,412.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	7,689.00
2. Foreign Travel Costs	3,384.00
Total Travel Cost	11,073.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	21,982.52
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.	Rabies prophylaxis	4,020.00
10.		
	Total Other Direct Costs	38,602.52

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	163,087.52

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	163,087.52	105,256.69

Total Indirect Costs 105,256.69

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	268,344.21

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	268,344.21

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 2** **Start Date:** 12/01/2019 **End Date:** 11/30/2020

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.60			6,479.00	1,747.00	8,226.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 85,202.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00	0.00	24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel 24,782.00

Total Salary, Wages and Fringe Benefits (A+B) 109,984.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	2,316.00
2. Foreign Travel Costs	8,245.50
Total Travel Cost	10,561.50

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	17,976.52
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.	Rabies prophylaxis	4,020.00
10.		
	Total Other Direct Costs	34,596.52

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	155,142.02

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	155,142.00	100,128.65

Total Indirect Costs 100,128.65

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	255,270.67

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	255,270.67

L. Budget Justification

(Only attach one file.) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 3** **Start Date:** 12/01/2020 **End Date:** 11/30/2021

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.80			8,639.00	2,329.00	10,968.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 87,944.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00	0.00	24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel: 24,782.00

Total Salary, Wages and Fringe Benefits (A+B): 112,726.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	6,118.00
2. Foreign Travel Costs	3,384.00
Total Travel Cost	9,502.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	17,976.52
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.	Rabies prophylaxis	4,020.00
10.		
	Total Other Direct Costs	34,596.52

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	156,824.52

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	156,824.52	101,214.21

Total Indirect Costs 101,214.21

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	258,038.73

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	258,038.73

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 4

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 4** **Start Date:** 12/01/2021 **End Date:** 03/31/2022

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.40			4,319.00	1,165.00	5,484.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	6.00			30,502.00	7,986.00	38,488.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 43,972.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						

Total Number Other Personnel

Total Other Personnel

Total Salary, Wages and Fringe Benefits (A+B) 43,972.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	4,167.00
2. Foreign Travel Costs	
Total Travel Cost	4,167.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	2,163.43
2.	Publication Costs	6,000.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.		
9.		
10.		
	Total Other Direct Costs	8,163.43

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	56,302.43

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	56,302.43	36,337.50

Total Indirect Costs 36,337.50

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	92,639.93

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	92,639.93

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		305,748.00
Section B, Other Personnel		74,346.00
Total Number Other Personnel	9	
Total Salary, Wages and Fringe Benefits (A+B)		380,094.00
Section C, Equipment		
Section D, Travel		35,303.50
1. Domestic	20,290.00	
2. Foreign	15,013.50	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		115,958.99
1. Materials and Supplies	60,098.99	
2. Publication Costs	6,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	37,800.00	
9. Other 2	12,060.00	
10. Other 3		
Section G, Direct Costs (A thru F)		531,356.49
Section H, Indirect Costs		342,937.05
Section I, Total Direct and Indirect Costs (G + H)		874,293.54
Section J, Fee		
Section K, Total Costs and Fee (I + J)		874,293.54

Re: [EXTERNAL] PREEMPT - A few important items

Tonie Rocke (b) (6) @gmail.com>

Fri 3/23/2018 3:46 PM

To: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>
Cc: Luke Hamel <hamel@ecohealthalliance.org>; Richgels, Katherine L <krichgels@usgs.gov>; Rocke, Tonie E <trocke@usgs.gov>; Jonathon Musser <musser@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>

Hello all: I sent my budget justification to Ana several days ago. Thanks for addressing safety issues Rachel!

Sent from my iPhone

On Mar 23, 2018, at 2:38 PM, Abbott, Rachel <rabbott@usgs.gov> wrote:

> Hi Luke,
> I have added some paragraphs to the page you sent. It just deals with safety of RCN, so I hope that is enough. Most of the text came out of documents we have to write to get approval to use our RCN vaccines in the field (risk analysis for USDA CVB and environmental assessment for USGS). Unfortunately, as I said before, I'll be unavailable until next Thursday, but Tonie should be back in her office on Monday.
> --Rachel
>
> On Fri, Mar 23, 2018 at 1:45 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>> Hi Rachel and Katherine,
>>
>> I wanted to address a few important PREEMPT items with you:
>>
>> - Regarding **NWHC budget justification**
>>   - If you have not already done so (and I apologize for not knowing the answer), could you please send us your budget justification document? We are hoping to have all collaborator budgets and budget justifications as soon as possible.
>> - Regarding language on **'Long-term safety and efficacy'**
>>   - In the PREEMPT proposal, we must state how we will establish methods to assess the 'long-term safety and efficacy of our preemptive approaches'
>>   - Given your field of work, do you have any existing language on how to address potential negative impacts of intervention approaches on non-target species?
>>   - I have attached language from the BAA to provide you with further guidance on what DARPA requires us to include.
>>   - This being said - Rachel and Katherine, could you please write-up a short section (a paragraph or so), that addresses this issue 'long-term safety and efficacy'?
>>   - I apologize for the extremely short notice, but **we would greatly appreciate it if you could return this to us by tomorrow afternoon, Sat. (3/24).**
>> - Regarding '**pricing assumptions'** for NWHC facilities
>>   - Previously, we had asked you to identify any 'pricing assumptions' that may correspond with use of government facilities. Due to confusion about

what exactly was being asked for, we reached out to DARPA staff, asking them to clarify the matter:

- We asked: "EcoHealth Alliance has a USG entity listed as a subcontractor in our proposal. Is the USG entity required to identify any pricing assumptions beyond those within their fully detailed and documented budget?
- To which they responded: "No"
  - Long story short...there is NO need for you to identify any additional pricing assumptions.

Thank you and please let me know if you have any questions. I will be available by email and phone (mobile number listed below) over the weekend, should you need to contact me.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

--
Rachel Abbott
USGS National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711
(608) 270-2489
Fax: (608) 270-2415

<PREEMPT_Eco_Impacts_Risk_Plan RCA.docx>

Re: [EXTERNAL] PREEMPT - A few important items

Luke Hamel <hamel@ecohealthalliance.org>

Fri 3/23/2018 3:51 PM

To: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>
Cc: Richgels, Katherine L <krichgels@usgs.gov>; Rocke, Tonie E <trocke@usgs.gov>; Jonathon Musser <musser@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>

Hi Rachel and Katie,

Thank you for agreeing to take on that section. Regarding the budget justification, I have attached a template with appropriate headings and language that is already correctly formatted. **I would just ask you to insert the appropriate name/cost amount, substituting CAPITALIZED words and filling in gaps (indicated by underscores).**

Each section in the budget (e.g. Personnel, fringe, travel, etc.) should have a corresponding section in the budget justification (as shown in the template). Essentially, any line item that is listed in the budget needs to be justified in the 'budget justification' document.

Whatever information you could fill in would be extremely helpful. If you feel unsure about certain sections, or don't feel as though you have the time to complete the entire document, we are happy to complete any remaining sections. We just ask that you send us whatever progress you've made, by Sunday afternoon (3/25). My apologies for requesting this with the weekend fast approaching.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(b) (6) (mobile)
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On Fri, Mar 23, 2018 at 3:19 PM, Abbott, Rachel <rabbott@usgs.gov> wrote:

> Hi Luke,
> I will write the paragraph on long-term safety and efficacy and send it to you later this afternoon (I'll be on vacation for a few days starting tomorrow).

I don't know what the budget justification document is and only have budget files that Tonie has already sent to you. I have attached them here. If it is a specific form we need to fill out, Katie might be the best person to enter the required information.
--Rachel

On Fri, Mar 23, 2018 at 1:45 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

Hi Rachel and Katherine,

I wanted to address a few important PREEMPT items with you:

- Regarding **NWHC budget justification**
  - If you have not already done so (and I apologize for not knowing the answer), could you please send us your budget justification document? We are hoping to have all collaborator budgets and budget justifications as soon as possible.
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  - In the PREEMPT proposal, we must state how we will establish methods to assess the 'long-term safety and efficacy of our preemptive approaches'
    - Given your field of work, do you have any existing language on how to address potential negative impacts of intervention approaches on non-target species?
    - I have attached language from the BAA to provide you with further guidance on what DARPA requires us to include.
    - This being said - Rachel and Katherine, could you please write-up a short section (a paragraph or so), that addresses this issue 'long-term safety and efficacy'?
      - I apologize for the extremely short notice, but **we would greatly appreciate it if you could return this to us by tomorrow afternoon, Sat. (3/24).**
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  - Previously, we had asked you to identify any 'pricing assumptions' that may correspond with use of government facilities. Due to confusion about what exactly was being asked for, we reached out to DARPA staff, asking them to clarify the matter:
    - We asked: "EcoHealth Alliance has a USG entity listed as a subcontractor in our proposal. Is the USG entity required to identify any pricing assumptions beyond those within their fully detailed and documented budget?
    - To which they responded: "No"
      - Long story short...there is NO need for you to identify any additional pricing assumptions.

Thank you and please let me know if you have any questions. I will be available by email and phone (mobile number listed below) over the weekend, should you need to contact me.

Best,

Luke Hamel
Program Assistant

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--
Rachel Abbott
USGS National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711
(608) 270-2489
Fax: (608) 270-2415

Budget Justification Template (Please follow the guidance in this template for each budget period)

PHASE 1

BASE PERIOD 1

A. Personnel ($Total)

- **NAME**, **PhD**, TITLE, will oversee all aspects….has in-depth knowledge and experience in…with expertise in…. We request $AMOUNT p.a. salary for NAME, who will dedicate # months p.a. to this project for phase ___ years ____.
- **NAME, PhD, TITLE**, will guide and advise…has worked with emerging zoonoses for over…. has experience managing…. We request $AMOUNT p.a. salary for NAME who will dedicate # months p.a. on this project for phase ____ years ____.
- **Post-doctoral fellow (TBD),** will help lead and coordinate all field and laboratory activities as well as data analyses and will dedicate # months p.a. to this project. We request $AMOUNT p.a. to cover stipend for a 3 year fellowship award.

B. Fringe ($Total)

Fringe benefits are calculated as per INSTITITION federally negotiated rate of ___% of base salary per year.

C. Travel ($Total)

Domestic Travel ($Total): We are requesting $AMOUNT in phase___ year___ to support domestic travel from ____to ____ for one (1) Co-PI/Co-I **and** one (1) research scientist to attend the **_____ meeting**. We calculate the expenses per person as follows: 2 economy, round-trip tickets (departure location <> return location) at $AMOUNT/person, # nights in hotel at $AMOUNT/person, and a total per diem allowance of $AMOUNT/person.

We are requesting $AMOUNT in the phase____year____ to support domestic travel from ____, to ____, for one (1) Co-PI/Co-I **and** one (1) research scientist to attend ____ meeting. We calculate the expenses per person as follows: 2 economy, round-trip tickets (departure <> return) at $AMOUNT/person, # nights in hotel at $AMOUNT/person, and a total per diem allowance of $AMOUNT/person.

International Travel ($Total): We request $AMOUNT p.a. for phase____ years____ to support international travel from Departure location to project study regions in LOCATION. We have budgeted for either a) one (1) Co-PI **and** two (2) research scientist; **or** b) three (3) research scientists to travel three

times to each region for ____ with local partners. Expenses per person for each trip are calculated as follows: 2 economy round trip tickets (ldeparture location<> return location) at $AMOUNT each, lodging at $AMOUNT x # nights, a total per diem allowance of $AMOUNT. Total estimated travel expenses to location per person per trip are $AMOUNT.

D. Field work ($Total)

Field team ($Total): We requesting $AMOUNT per year for phase____ years___ and $AMOUNT for phase___ year____ to cover stipends for # field assistants to conduct biological sampling.

Field visits ($Total): We are requesting $AMOUNT p.a. for phase___ years____and $AMOUNT for phase____ year____ to cover transportation to field sites.

E. Supplies and Materials ($Total)

We are requesting a total of **$AMOUNT** for supplies and materials across all phases and years. Expenses are calculated as follows:

- **Biological sampling supplies ($Total)** We are requesting $AMOUNT for phase___ year____ and $AMOUNT for phase____ year____ to purchase necessary supplies for biological sampling including (type, examples of) materials necessary for the collection (e.g. vials, swabs) and transportation of biological samples, and microscopes.
- **Computing devices ($Total)** 2 laptop computers at $AMOUNT for research analyses.
- **Office supplies ($Total):** We are requesting $AMOUNT to purchase office supplies to record biodiversity and laboratory data (notebooks, clipboards, pens, etc.).
- **Database development ($Total):** We request $AMOUNT for the development of an extensive, comprehensive database to store all collected data.
- **Publications ($Total):** We are requesting $AMOUNT p.a. for phase____ years____ to support journal publication costs of research results. We expect to produce ____ publications per year.
- **Internet ($Total):** Internet service for ___ months per year at $40.00 per month.
- **Cellphone service ($Total):** Cellphone service for ___ months per year at $AMOUNT per month.
- **Google apps for work ($Total):** Google apps service for __ months per year at $AMOUNT per month.
- **Printing and Photocopying ($1,620):** Printing and photocopying of survey instrument, survey guide and training materials.

F. Equipment ($Total)

We request a total of $AMOUNT in phase____ year____ to purchase ____ at $AMOUNT and ____ at $AMOUNT to preserve samples prior to shipment.

H. Indirect Costs ($Total)

We are requesting a federally negotiated indirect cost rate of ____% on all direct costs.

PHASE 1

BASE PERIOD 2

PHASE 2

OPTION PERIOD 1

PHASE 2

OPTION PERIOD 2

[EXTERNAL] Scheduling a PREEMPT call for Mon. (3/26)

Luke Hamel <hamel@ecohealthalliance.org>

Fri 3/23/2018 3:57 PM

To: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>
Cc: Dr. Peter Daszak <daszak@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>; Alison Andre <andre@ecohealthalliance.org>

Hi Rachel and Katie,

Could you please look at Tonie's calendar and see if she is available for a PREEMPT call on Mon. (3/26)? Please use this link to select which time(s) she is likely available.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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[EXTERNAL] Re: Project DEFUSE proposal

Luke Hamel <hamel@ecohealthalliance.org>

Sat 3/24/2018 9:43 AM

To: Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>
Cc: Rocke, Tonie E <trocke@usgs.gov>; Kateri.Paul@parc.com <Kateri.Paul@parc.com>; Dr. Peter Daszak <daszak@ecohealthalliance.org>; William B. Karesh <karesh@ecohealthalliance.org>

Excellent. Thank you very much, Jerome!

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
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On Fri, Mar 23, 2018 at 9:36 PM, <Jerome.Unidad@parc.com> wrote:

> Peter, Luke and the rest of the EHA team (CC: Tonie Rocke),
>
> Please find attached an edited version of the DEFUSE proposal that includes additional text from PARC. I included an additional figure (Fig. 15, also included here in .pptx), two new references marked with comments in our 2-3 paragraphs on the spray technology and inserted us in some of the other relevant sections. I also included a paragraph discussing PARC's commercialization strategy for FEA. Feel free to incorporate this in a table/timeline form, as needed – to identify clear milestones for Technology Transition.
>
> If you need further details on any of these, I'd be happy to oblige.
>
> Best,
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Jerome.Unidad@parc.com
Sent: Friday, March 23, 2018 6:37 PM
To: hamel@ecohealthalliance.org
Cc: trocke@usgs.gov; Kateri.Paul@parc.com; daszak@ecohealthalliance.org; karesh@ecohealthalliance.org
Subject: [EXTERNAL] Project DEFUSE proposal
Attachments: fig15.pptx; DARPA PREEEMPT DEFUSE full v2 JU 032318.docx

Peter, Luke and the rest of the EHA team (CC: Tonie Rocke),

Please find attached an edited version of the DEFUSE proposal that includes additional text from PARC. I included an additional figure (Fig. 15, also included here in .pptx), two new references marked with comments in our 2-3 paragraphs on the spray technology and inserted us in some of the other relevant sections. I also included a paragraph discussing PARC's commercialization strategy for FEA. Feel free to incorporate this in a table/timeline form, as needed – to identify clear milestones for Technology Transition.

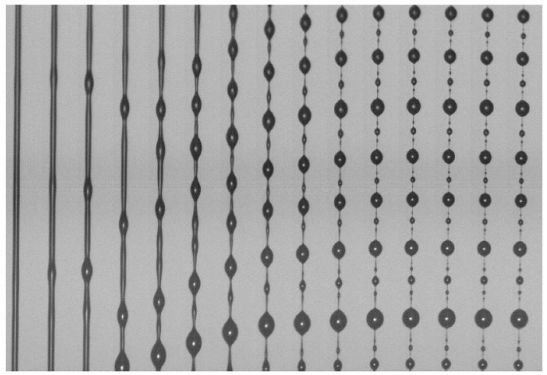
If you need further details on any of these, I'd be happy to oblige.

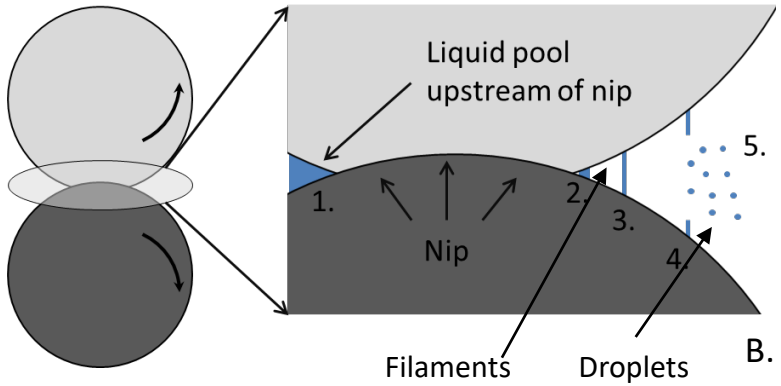
Best,

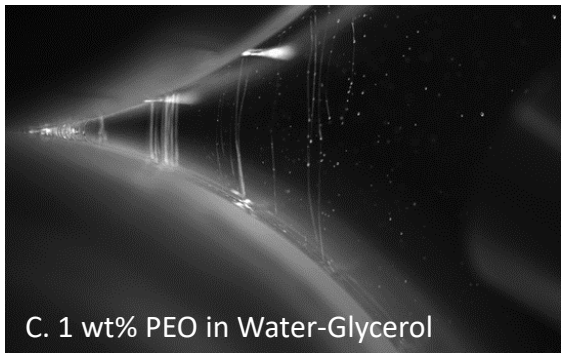
Jerome

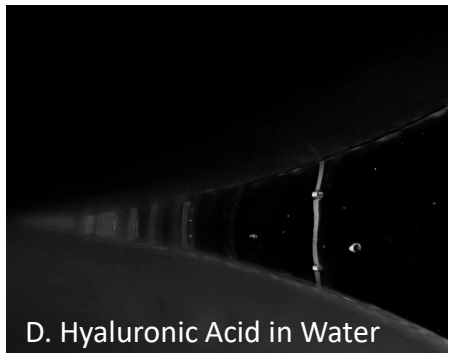
Jerome Unidad, PhD
Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory
PARC, A Xerox Company

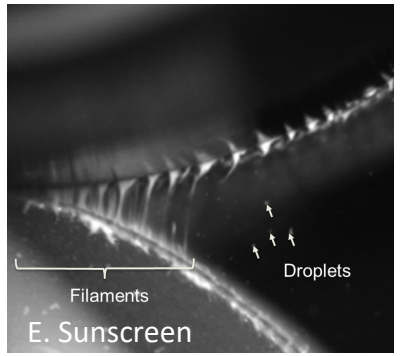
A.

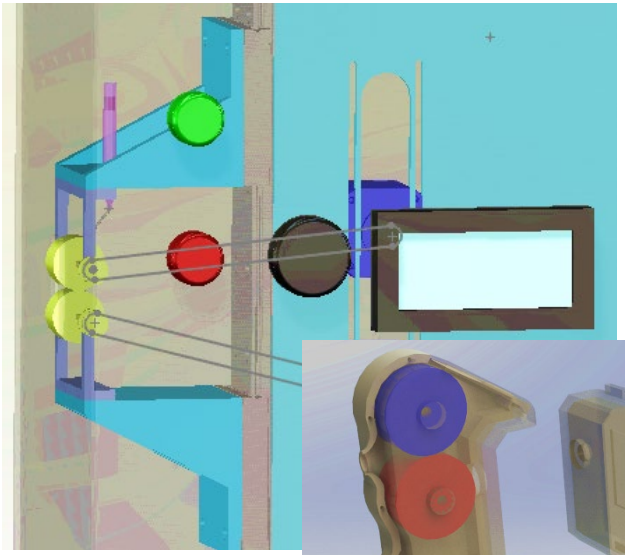


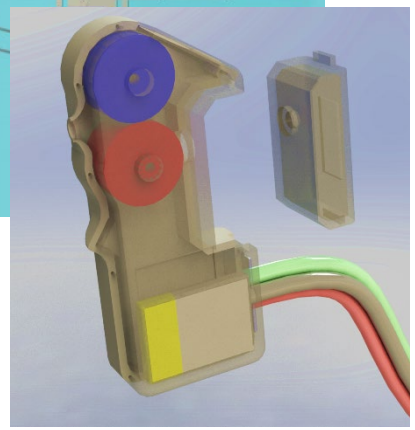












F. Different Form Factors for FEA Prototype

A. EXECUTIVE SUMMARY

Technical Approach: Our goal is to defuse the potential for spillover of novel bat-origin high-zoonotic risk SARS-related coronaviruses in Southeast Asia. **In TA1** we will develop **host-pathogen ecological niche models** to predict the species composition of bat caves across Southeast Asia. We will parameterize this with a full inventory of host and virus distribution at our field sites, three caves in Yunnan Province, China and a series of unique datasets on bat host-viral relationships. By the end of Y1, we will use these to create a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens at any site across Asia. We will intensively sample bats at our field sites to sequence SARSr-CoV spike proteins, reverse engineer them to conduct binding assays, and insert them into SARS-CoV backbones to infect humanized mice to assess capacity to cause SARS-like disease. Our modeling team will use these data to build **machine-learning genotype-phenotype models** of viral evolution and spillover risk. We will uniquely validate these with human serology data through LIPS assays designed to assess which spike proteins allow spillover into people.

In TA2, we will evaluate two approaches to reduce SARSr-CoV shedding in cave bats: (**1) Broadscale Immune Boosting**, in which we will inoculate bats with immune modulators to upregulate their innate immune response and downregulate viral replication; **(2) Targeted Immune Priming**, in which we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance innate immunity against specific, high-risk viruses. We will trial inoculum delivery methods on captive bats including automated aerosolization, transdermal nanoparticle application and edible, adhesive gels. We will use stochastic simulation modeling informed by field and experimental data to characterize viral dynamics in our cave sites, to maximize timing, inoculation protocol, delivery method and efficacy of viral suppression. The most effective delivery method and treatments will be trialed in our experimental cave sites in Yunnan Province, with reduction in viral shedding as proof-of-concept.

Management Approach: Members of our collaborative group have worked together on bats and their viruses for over 15 years. The lead organization, EcoHealth Alliance, will oversee all modeling, lab, and fieldwork. EHA staff will develop models to evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for delivery of both immune boosting and immune targeting inocula. Specific work will be subcontracted to the following organizations:

- Prof. Ralph Baric, UNC, will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades.
- Prof. Linfa Wang, Duke-NUS, will lead work on immune boosting, building from his groups' pioneering work on bat immunity.

- Dr. Zhengli Shi, Wuhan Institute of Virology will conduct viral testing on all collected samples, binding assays and some humanized mouse work.
- Dr. Tonie Rocke, USGS National Wildlife Health Center will develop a delivery method for immunological countermeasures, following from her work on vaccine delivery in wildlife, including bats.
- Dr. Jerome Unidad, PARC will develop an innovative aerosol technology that could work with a wide-range of formulations into a field-deployable device that can be used for large-scale inoculation of bats.

B. EXECUTIVE SUMMARY SLIDE

;lkj;lkj;lkj;lkj;lkj
;klj;lkj;lk
;lj;lkj;lkj
;lkj;lkj
;lkj;lkj

C. GOALS AND IMPACT

Overview

The overarching goals of DEFUSE are:

- **Identify and model the spillover risk** of novel SARS-related CoVs in South and SE Asia
- Design and demonstrate proof-of-concept that interventions to upregulate the naturally low innate immunity of bats to viruses (**immune boosting**) and to high risk SARSr-CoVs in particular (**immune priming**) will transiently reduce spillover risk.

We will analyze, design and field-test a novel strategy to reduce risk of viral emergence from bats that will help protect the warfighter within SACOM and SEACOM, and will be scalable to other systems including Ebola virus, rabies and other bat-origin pathogens.

Innovation and uniqueness:

Bats harbor more emerging zoonoses than any other group of mammals, and are ubiquitous, abundant, wide-ranging and often overlooked. Despite this, other than PPE, there is no available current technology to reduce the risk of exposure to novel coronaviruses from bats. Models of bats' capacity to harbor viruses, of ecological and environmental drivers of their emergence, and of the evolutionary potential of different strains to spillover are rudimentary. No vaccines or therapeutics exist for SARSr-CoVs, and exposure mitigation strategies are non-existent. SARSr-CoVs are enzootic in Asian, African[1], and European bats[2] that roost in caves but forage widely at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have recently shown evidence of spillover of SARSr-CoVs into people in China, unrelated to the original SARS pandemic, and have isolated strains capable of producing SARS-like disease in humanized mice that don't respond to antibody treatment or vaccination. These viruses are a clear-and-present danger to our military and to global health security because of their continuous circulation and evolution in bats and periodic spillover into humans in locations where surveillance is virtually nonexistent.

EcoHealth Alliance leads the world in predictive models of viral emergence. We will build on our machine-learning models of spillover hotspots, host-pathogen ecological niche and genotype-phenotype mapping by incorporating unique datasets to validate and refine hotspot risk maps of viral emergence in SE Asia and beyond. We have shown that bats are able to carry otherwise lethal viruses by virtue of dampened innate immunity (e.g. inflammatory) pathways, which likely evolved as an adaptation to the physiologic stress of flight. We will use this insight to design strategies, like small molecule Rig-like receptor (RLR) or Toll-like receptor (TLR) agonists, to upregulate bat immunity and down-regulate viral replication in their cave roosts, thereby significantly reducing the frequency and magnitude of viral shedding and spillover **(broadscale immune boosting strategy)**. We will complement this by treating bats with novel chimeric polyvalent recombinant spike proteins to enhance their adaptive immune response against specific, high-risk coronaviruses **(targeted immune priming strategy)**, especially when their innate immune response is boosted as above. We will design novel automated application methods, based on our previous work delivering wildlife vaccines, to apply these interventions in a way that eliminates the need for a person to enter a cave and potentially get exposed to bat borne viruses or other hazards.

Technical Area 1

Our strategy to reduce spillover risk of bat SARS-related CoVs begins with modeling to predictively assess spillover risk across South and SE Asia using baseline genotype-phenotype analysis of host and strain diversity from the literature, from surveillance in our designated model caves in China, and across the region in other projects. In TA1, the DEFUSE modeling and analytics team, will build joint species distribution models (JSDM) of environmental and

ecological correlates and traits of cave bat communities to predict species composition of bat caves across Southern China, South and SE Asia. Dr. Epstein at EHA will coordinate animal experimental work with the teams at NWHC, Duke-NUS and Wuhan and radio telemetry studies with the field surveillance team. We will then use a series of datasets we have built to produce host-virus risk models for the region. These include our comprehensive database of bat host-viral relationships and estimates of zoonotic viral richness per bat species[3]; biological inventory data on all bat caves in Southern China; and modeled species distribution data for all bats. We will parameterize the model with data from three cave sites in Yunnan, China (one with high-risk SARSr-CoVs, two other control/comparison sites), including: radio- and GPS-telemetry to identify home range and additional roost sites for each bat species; inventory of bat population density, distribution and segregation and their daily, weekly and seasonal changes; viral prevalence and individual viral load; shedding of low- and high-risk SARSr-CoV strains among bat species, age classes, genders; and telemetry and mark-recapture data to assess metapopulation structure and inter-cave connectivity. We will test and validate model predictions of a cave's viral spillover potential with data from prior PREDICT sampling in 7 other Asian countries. At the end of Yr 1, we will produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens in a region. **The 'Spatial viral spillover risk' app** will be updated real-time with surveillance data (e.g. field-deployable iPhone and android compatible echolocation data) from our project and others, to ground-truth and fine-tune its predictive capacity.

The Wuhan Institute of Virology team will test bat fecal, oral, and blood samples for SARSr-CoVs. We will collect viral load data using fresh fecal pellets from individually sampled bats and from tarps laid on cave floors deployed where necessary to reduce roost disturbance. SARSr-CoV spike proteins will be sequenced, analyzed phylogenetically for recombination events, and high-risk viruses (spike proteins close to SARS-CoV) characterized and isolated. The UNC team will reverse-engineer spike proteins to conduct binding assay to human ACE2 (the SARS-CoV receptor). They will culture SARS-like bat coronaviruses to distinguish high-risk strains that can replicate in primary human cells and low risk strains that require exogenous enhancers. Viral spike glycoproteins that bind receptors will be inserted into SARS-CoV backbones, inoculated into human cells and humanized mice to assess capacity to cause SARS-like disease, and to be blocked by monoclonal therapies, the nucleoside analogue inhibitor GS-5734[4] or vaccines against SARS-CoV[4-8].

The EHA modeling team will use these data to **build models of risk of viral evolution and spillover**. These genotype-to-phenotype machine-learning models will predict viral ability to infect human host cells based on genetic traits and results of receptor binding and mouse infection assays. Using data on diversity of spike proteins, recombinant CoVs, and flow of genes within each bat cave via bat movement and migration, we will estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection.

Finally, virus-host relationship and bat home range data will be used to estimate spillover potential - extending models well beyond our field sites. We will then **validate model predictions of viral spillover risk** by **1)** conducting spike protein-based binding and cell culture experiments, and **2)** identifying spillover strains in people near our bat cave sites. Our preliminary work on this shows ~3% seroprevalence to SARSr-CoVs, using a specific ELISA [REF]. We will design LIPS assays to the specific high- and low- zoonotic-risk SARSr-CoVs identified in this project as we have done previously [REF]. We will use previously collected and newly collected human sera from these populations to test for presence of antibodies to the high- and low-risk SARSr-CoVs identified by our modeling. We will then **model optimal strategies to maximize treatment efficacy** for TA2, using stochastic simulation modeling informed by field and experimental data to characterize viral circulation dynamics in bats. We will estimate frequency and population coverage required for our intervention approaches to suppress viral spillover. We will determine the seasons, locations within a cave, and delivery methods (spray, swab, or automated cave mouth or drone) that will be most effective. Finally we will determine the time period treatment will be effective for, until re-colonization or evolution leads to return of a high-risk SARSr-CoV.

Technical Area 2

In TA2, we will **develop scalable approaches that target and suppress the animal virus in its reservoir(s)and/or vector(s), to reduce the likelihood of virus transmission into humans**. We will evaluate two approaches to defuse SARS-related CoV spillover potential**: 1) Broadscale Immune Boosting:** using the unique immune damping in bats that our group has discovered, we will apply immune modulators like bat interferon to live bats, to up-regulate their naïve immunity and then assess their ability to suppress viral replication and shedding; **2) Targeted Immune Priming:** building on preliminary development of polyvalent chimeric recombinant SARSr-CoV spike proteins, we will conduct application trials with live bats to assess suppression of replication and shedding of a broad range of dangerous SARS-related CoVs.

Both lines of work will begin in Yr 1 and run parallel. Prof. Linfa Wang (Duke-NUS) will lead the immune boosting work, building on his pioneering work on bat immunity[9] which shows that the long-term coexistence of bats and their viruses has led to equilibrium between viral replication and host immunity. This is likely due to down-regulation of their innate immune system as a fitness cost of flight[9]. The weakened functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may allow bats to maintain an effective, but not over-response to viruses[10]. A similar finding was observed for bat IFNA, which is less abundant but constitutively expressed without stimulation[11]. Given high native SARSr-CoV load in bats, we aim to boost bat innate immunity through the IFN pathway, break the host-virus equilibrium to suppress bat SARSr-CoV replication and shedding.

We will trial the following, concurrently and competitively, for efficiency, cost and scalability: **i)** Universal bat interferon. Aerosol spraying or intranasal application of IFN or other small molecules reduces viral loads in humans, ferrets and mouse models[12,13]. Interferon has been used clinically when antiviral drugs are unavailable, e.g. against filoviruses[14]. Replication of SARSr-CoV is sensitive to interferon treatments, as shown in our previous work[13] ; **ii)** Boosting bat IFN by blocking bat-specific IFN negative regulators. Uniquely, bat IFNA is naturally constitutively expressed but cannot be induced to a high level[11], indicating a negative regulatory factor in the bat interferon production pathway. We will use CRISPRi to identify the negative regulator and then screen for compounds targeting this gene; **iii)** Activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7-dependent pathways. Our work showing that mutant bat STING restores antiviral functionality suggests these pathways are important in bat-viral coexistence[10]. By identifying small molecules to directly activate downstream of STING, we will activate bat interferon and promote viral clearance. A similar strategy will be applied to ssRNA-TLR7-dependent pathways; **iv)** Activating functional bat IFN production pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been demonstrated in a mouse model for SARS-CoV, IAV and HBV[12,15]; **v)** Inoculating crude coronavirus fragments to upregulate innate immune responses to specific CoVs – a partial step towards the targeted immune priming work below.

Prof. Ralph Baric (UNC) will lead the immune priming work. He will develop recombinant chimeric spike-proteins[16]from our known SARSr-CoVs, and those we characterize during project DEFUSE. The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain[17]. In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles[6,18-21]. We will test these in controlled lab conditions, taking the best candidate forward for testing in the field. We have built recombinant spike glycoproteins harboring structurally defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broad scale immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, potentially reducing disease risk (and transmission risk to people) in these animals for longer periods[22,23].

The immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established the only experimental breeding colony of cave bats (*Eonycteris spelaea*) in SE Asia. This genus is evolutionarily related to *Rhinolophus* spp. (the hosts of SARSr-CoVs), so we have confidence that results will be transferable. Our initial proof-of-concept tests will be in this experimental colony, extended to a small group of wild-caught *Rhinolophus*

sinicus bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with *Rhinolophus* sp. bats in the BSL-4 facility at CSIRO, AAHL (L.Wang, unpublished results).

Finally, work on a delivery method for our immune boosting and priming molecules will be developed and implemented by Dr. Tonie Rocke at the USGS, National Wildlife Health Center who has previously developed animal vaccines through to licensure[24]. Using locally acquired insectivorous bats[25,26], we will assess delivery vehicles and methods including: 1) transdermally applied nanoparticles; 2) series of sticky edible gels that bats will groom from themselves and each other; 3) aerosolization via sprayers that could be used in cave settings; 4) automated sprays triggered by timers and movement detectors at critical cave entry points, and 5) sprays delivered by remote controlled drone. We have already used simple gels to vaccinate bats against rabies in the lab[25], and hand delivered these containing biomarkers to vampire bats in Peru and Mexico to show they are readily consumed and transferred among bats. In our bat colony, we will trial delivery vehicles using the biomarker rhodamine B (which marks hair and whiskers upon consumption) to assess uptake. The most optimal approaches will then be tested on wild bats in our three cave sites in Yunnan Province with the most successful immunomodulators from TA2. Fieldwork will be conducted under the auspices of Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance). A small number of bats will be captured and assayed for viral load and immune function after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has had unique access to these sites for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for experimental trials from the Provincial Forestry Department. We expect to be successful, as we have worked with the Forestry Department collaboratively for 10 years, with support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife. EHA has a proven track record of rapidly obtaining IACUC and DoD ACURO approval for bat research.

Deliverables:

- App identifying geographical risk of spillover for novel SARSr-CoVs in SE Asia
- Identified indicators (modeled and validated) of spillover capacity for different viral strains.
- Proven mechanistic approach to modulating bat innate immunity to reduce viral shedding
- Tested and validated delivery mechanism for bat cave usage including vaccines in other bat host-pathogen systems (e.g. rabies, WNS).
- Proof-of-concept approach to transiently reducing viral shedding in wild bats that can be adapted for other systems including Ebola virus.

D. TECHNICAL PLAN

Technical Area I:

Choice of site and model host-virus system. For the past 14 years, our team has conducted coronavirus surveillance in bat populations across Southern China, resulting in <150 CoV identifications in ~10,000 samples[27-29]. Bat SARSr-CoVs are genetically diverse, especially in the S gene, and most are highly divergent from SARS-CoV. However, in a cave site complex in Yunnan Province, we have found bat SARSr-CoVs with S genes extremely similar to SARS-CoV, and which, as a quasispecies population assemblage contain all the genetic components of epidemic SARS-CoV[30].

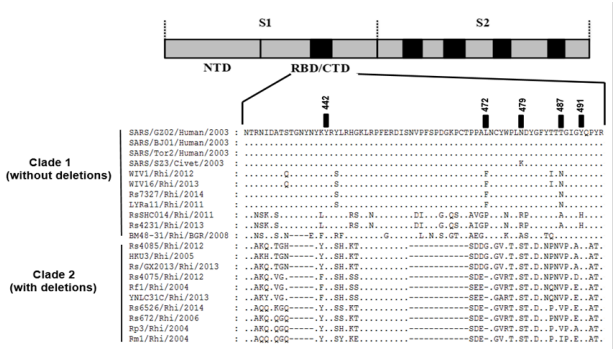


Fig. 1: Alignment of amino acid sequence of the receptor-binding motif in the spike protein of SARSr-CoVs and SARS-CoV[30]. Numbered amino acid is the key residues which is responsible for SARS-CoV S and human ACE2 interaction[31].

We have isolated three strains at this site (WIV1, WIV16 and SHC014) that unlike other SARSr-CoVs, do not contain two deletions in the receptor-binding domain (RBD) of the spike, and share substantially higher sequence identity to SARS-CoV (Fig. 1). These viruses have been demonstrated to use human ACE-2 receptor for cell entry as SARS-CoV does (Fig. 2), and replicate efficiently in various animal and human cells[27,29,30,32,33] including primary human lung airway cells, similar to epidemic SARS-CoV[7,8].

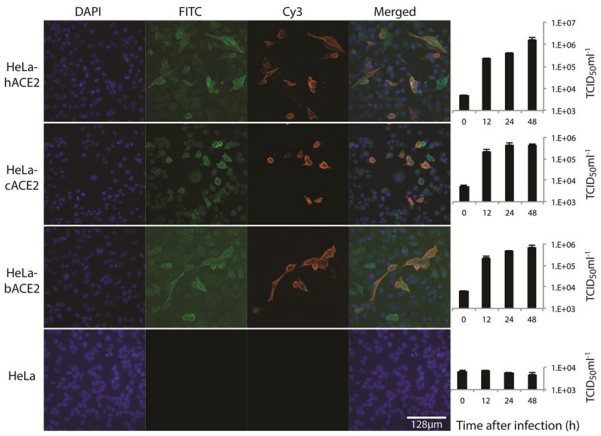


Fig. 2: Bat SARSr-CoV WIV1 replicates efficiently in HeLa cells expressing human, civet and bat ACE2[29].

Chimeras (recombinants) with these SARSr-CoV S genes inserted into a SARS-CoV backbone, as well as synthetically reconstructed full length SHCO14 and WIV-1 bat viruses cause SARS-like illness in humanized mice (a model that expresses human ACE2 receptor), with clinical signs that are not reduced by SARS-CoV monoclonal antibody therapy or vaccination[7,8]. We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort)[34], suggesting active spillover. These data, phylogeographic analysis of SARSr-CoVs (Fig. 3), and coevoutionary analysis of bats and their CoVs (unpubl. data), suggest that bat caves in SW

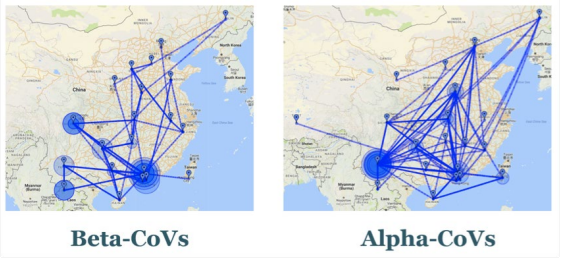


Figure 3. Ancestral location reconstruction for Beta- and Alpha-CoVs. The bigger the circle is, the more ancestral the corresponding node is.

China, and *Rhinolophus* spp. bats are the likely origin of the SARS-CoV clade, and **therefore a clear-and-present danger for the re-emergence of SARS-CoV or a similar pathogenic virus.** The *Rhinolophus* spp. bats that harbor these viruses occur throughout SE Asia, across S. and W. Asia. **Thus, the geographic focus of DEFUSE is to use our research at this site to reduce the risk for the warfighter of these viruses spilling over across the region (West, South and SE Asia).**

Spatial models of bat origin high-risk viruses across S and SE Asia. We will build models that predict regional-scale bat and viral diversity in cave sites across South and SE Asia to enable warfighters and planners to estimate regional-scale risk from viral spillover based on locations. This will provide preliminary assessments for areas requiring greater on-the ground risk characterization to target deployment of viral suppression technologies. These regional-scale joint species distribution models (JSDM) will predict the composition of bat communities in caves in South Southern China, South and SE Asia. JSDMs use environmental and habitat data to predict the distributions of many species simultaneously, producing more accurate predictions than individual, separate species predictions by explicitly modeling positive and negative interactions between species and hidden factors such as shared habitat preferences. We will use a stochastic feedforward neural network to implement JSDMs that has proven effective at making predictions across multiple scales, with incomplete observations (as occurs for bats and their viruses), and explicitly accounting for bat species co-occurrence driven by shared environmental responses or evolutionary processes[35]. We will fit our JSDM to biological inventory data on over 200 caves in the region[36], using a combination of climatic and topographic variables including physiologically relevant bioclimatic variables (BIOCLIM) drawn from public, open source data sets[37], as well as proxies for subterranean habitat such as ruggedness and habitat heterogeneity. We will refine these models using regional-scale environmental variables (land-use, distance to roads, forest cover, degree of human disturbance etc.) and cave-specific variables (cave length, availability of roosting area, entrance dimensions, cave complexity, microclimate etc.). Our previous work has shown that these factors are predictors of bat species presence/absence at a given site[38]. Remote-sensing data and physical models will be used to estimate cave structures and microclimates where they are not available from biological inventory studies. We will validate our regional-scale species models using independent occurrence estimates and observations[39,40], including our extensive database on bat species occurrence in Southeast Asia [REF].

We will extend our predictions of bat communities to predictions of zoonotic disease risk using our unique species-level database of all known bat host-viral relationships[3] (Fig. 4); our >1800 viral detections from >20,000 individual bat samples in China and 7 other Asian countries (NIAID and USAID PREDICT); and results as they become available from a new 5-year DTRA-CBEP grant for field and lab investigations to characterize bat CoV diversity in Western Asia (Turkey, Jordan, Georgia, Pakistan, and Arabian Peninsula – EHA, Olival) to extend the

geographic scope of our predictive models. We will use two strategies to predict presence of viruses at sites. Firstly, as a base case, we will assume that species have equal probability of carrying their known viral species across their range. Second, we will include viral species as additional outputs in our JSDM. We will fit this host-viral JSDM using data restricted to a smaller set of sites where both host species composition and viral detections are available. Based on performance of both models on hold-out data, we will determine which provides the best predictive power. For species composition and viral presence predictions, we will validate our models against a 20% validation subset of data that is held out for model validation, as well as data collected at our field sites in Task 3.

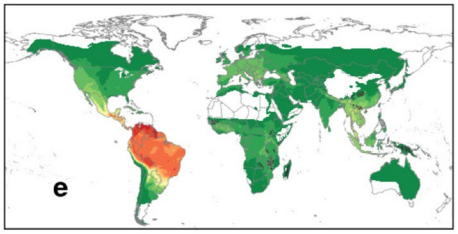


*Fig. 4: Predictive global map of total (known and unknown) viral diversity in bats (*Chiroptera *species). Based on EHA's unique database of all known mammal virus-host relationships[3].*

Prototype app for the warfighter. Drawing on experience building applications for data collection and analysis (e.g. https://flirt.eha.io/, https://eidr-connect.eha.io/, https://mantle.io/grrs), we will produce a prototype app for the warfighter that identifies the likelihood of dangerous viral pathogens spilling over from bats at a site. **The 'Viral spillover risk' app** will use outputs from our spatial risk modeling, data from EHA's extensive host-pathogen database, open-source species and pathogen ontologies, and app-directed crowd-sourced ultrasonic audio recordings to ground-truth and fine-tune its predictive capacity. This app will be updated in Y2 and Y3 to incorporate additional information on bat species-specific risk based on assays of host-virus binding and surveys of CoV prevalence. We will use risk-ranking algorithms developed by EHA (https://ibis.eha.io/) that use geolocation features, recency of information, and host and pathogen characteristics to display critical areas of high risk. The app will collect user GPS location data and preload bat species distribution and community composition estimates from our JSDMs. These will be refined with real-time surveillance data collected without the need to enter cave sites using field-deployable high-frequency microphones for bat detection[41]. We will combine reference acoustic calls from all bat species captured during proposed field work with existing data from bat call libraries globally to train species identification algorithms using bat echolocation call signatures. New algorithms using deep learning methods (e.g. convolutional neural networks[42]) will be developed, or adapted and externally validated on samples collected by the application to characterize bat species based on trained audio features. These models will be deployed on the mobile platform as they become available[42]. Bat species directly identified or estimated to occur within a scalable distance from the user will be automatically linked with viral diversity data from EHA's extensive host-pathogen database and with CoV sequence data from this project to deliver high-risk pathogen lists. The application will have 3 primary views; pathogens-

centric, bat-centric and map-centric. The pathogen-centric view will show a ranked list of likely pathogens in the user's current or selected location. The bat-centric view will show a ranked list of bat species for the user's location. The map-centric view will allow users to select a location for the other rank views, and will display a variety of map layers of interest, including heat map or distribution map layers profiling modeled or collected species occurrences around the user. Elements of the interface will be interactive, presenting popovers with more details when selected and displaying other map elements as appropriate. Alerts and notifications will give users a flexible way to monitor the app data passively, with the app proactively reaching out when critical information is received. The application will also offer a data collection module and accompanying interface elements to collect samples in the field and integrate collected data into the application database. The schemas, APIs, and protocols developed as part of this effort will be designed with principles of simplicity, interoperability, and usability in mind, including using RESTful URL schemes, and standardized data types and ontologies. Datasets will be hosted via cloud services from which the app will download updated information. Build and deployment processes will be reproducible, auditable, and transparent. All code modules will be continually available on EHA's GitHub page (LINK), be documented via README files in root directory of code repositories, and .zip archives containing code, datasets, and instructions for deployment will be made available. This will pave the way future incorporation of new structured biosurveillance data feeds and new species, viral, or host ontologies. This app will be designed for remote use (desktop platform) to assess specific sites in advance of personnel deployment on the ground, or in the field via mobile systems. This technology will improve overall situational awareness of existing and novel infectious agents found in bats, allowing DoD personnel to quickly identify areas that may pose the most significant risk for zoonotic spillover and rapidly deploy resources to respond to and mitigate their impact preemptively when necessary. The 'viral spillover risk' app will then be available to adapt for viral threats from other wildlife host species (e.g. rodents, primates) and ultimately for global use.

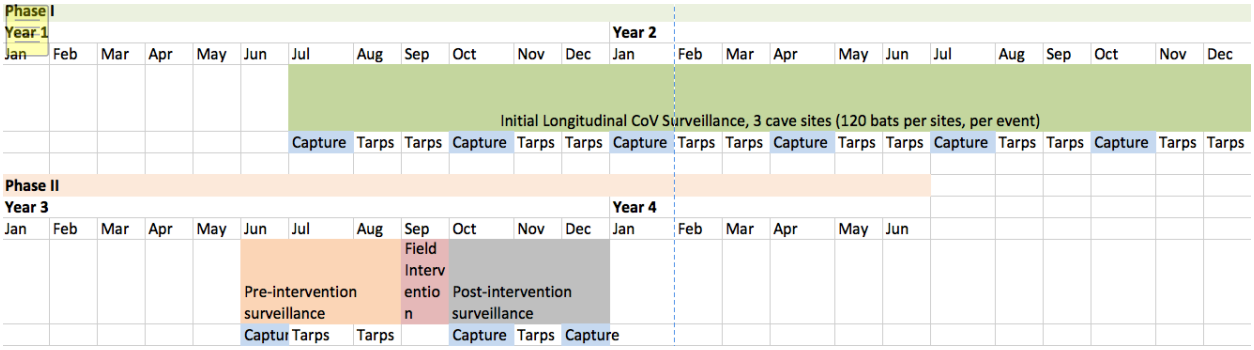
Full inventory of bat SARSr-CoV quasispecies at our cave test sites, Yunnan, China.
DEFUSE fieldwork will focus on three model cave test sites within a cave complex in Yunnan Province, SW China (MAP), where we have previously identified and isolated high-risk SARSr-CoVs able to infect human cells and cause SARS-like illness in mice[7,27,29,30]. At these sites, we will determine the baseline risk of SARSr-CoV spillover, prior to, during, and after our proof-of-concept field trials to reduce that risk. We will conduct longitudinal surveillance of bat populations to detect and isolate SARSr-CoVs, determine changes in viral prevalence over time, measure bat population demographics and movement patterns, to definitively characterize their SARSr-CoV host-viral dynamics. We will sample *Rhinolophus, Hipposideros*, and *Myotis* species, all of which carry SARSr-CoVs, and co-roost in the same caves[3,36]. Surveillance will be conducted before, during, and after deployment of our intervention field trial (Task X) to

establish baseline viral shedding detection rates and measure the impact of treatment on these. Field data will allow us to test the accuracy of our model predictions and compare the efficacy of laboratory trials in animal models with in-the-field trials.

Our test caves near Kunming, Yunnan Province, contain multiple co-roosting *Rhinolophus*, *Hipposideros*, and *Myotis* spp., although our preliminary data demonstrate that *R. sinicus* and *R. ferrumequinum* (which co-roost at our sites) are the SARSr-CoV primary reservoir, with *Hipposideros* and *Myotis* playing an insignificant role in viral dynamics. We will capture bats using harp traps and mist nets during evening flyout. Rectal, oral, and whole blood samples (×**2** per bat) will be collected for viral discovery using sterile technique to avoid cross-contamination. 2-mm wing tissue punch biopsies will be collected from each bat for host DNA bar-coding, sequencing of host ACE-2 receptor genes (interface site), and cophylogeny analyses. Standard morphological and physiological data will be collected for each bat (age class, sex, body weight, reproductive status etc.). In Phase I we will **sample 60** *Rhinolophus sinicus* and **60** *R. ferrumequinum*, our primary target species, (**120** bats total) **every three months** for non-lethal viral specimen collection over an **18 month period** of the project from all three cave sites. Given the average prevalence of SARSr-CoV in these species in our previous investigations in S. China (~6-9%, n=3304 *Rhinolophus* spp.), this sample size would enable to detect changes of 10% fluctuation in prevalence between sampling periods. Early in the sampling we will trial the efficacy of tarp collection of fresh feces and urine as a way of collecting viral dynamics data while reducing roost disturbance (REFS). To identify seasonal or reproductive cycle variation in viral dynamics, we will conduct repeated sampling of individuals and of tarps placed under the same roost site portion of a cave and examine roost-site fidelity (see below) to measure how well tarp-collected samples will track the general population. *Rhinolophus* species have a 7-week gestation period and generally give birth in the spring. Colony composition may change over the year, with bats aggregating during mating periods. These changes will affect viral dynamics and our sampling strategy will allow us to collect data over two mating and gestation periods and assess changes in viral prevalence. Additionally, we will conduct **pre-intervention (3 months prior to deployment) and post-intervention (3 months following deployment) CoV monitoring from these sites in Phase II (see Fig. X -Gantt chart)** to assess efficacy of our field intervention deployment. During months without physical bat trapping (2 months each quarter of sampling), fresh fecal pellets will be collected by placing clean polyethylene sheets measuring 2.0m x 2.0m beneath roosting bats. We will use infrared spotlights and digital infrared imaging to record the number and species of individuals above each plastic sheet. Fecal pellets may also be genetically barcoded to confirm species identification[43] as we routinely do for other bat surveillance projects. All specimens will be preserved in viral transport medium and immediately frozen in liquid nitrogen dry shippers in the field, then transported to partner laboratories with maintained cold chain and strict adherence to biosafety protocols. Each bat will be marked with a subcutaneous microchip (PIT tag) containing

a unique ID number (**see below**). **Study caves and bat roosts will be surveyed using portable LiDAR technology**[44-46], to give a 3-D image of the roost area which will provide data on species composition and volume/surface area that needs to be covered when applying the immune treatments in TA2 (Fig. XX). We will adjust individual sampling quotas per species to optimize viral detection based on host-specific prevalence of previous and ongoing host-pathogen models, as well as ongoing lab results from bat sampling.

Our team has more than 30 years of collective experience in safe and humane handling of bats for biological sampling. This project will operate under appropriate IACUC/ACURO and PPE guidelines. EHA has several ongoing DTRA-supported projects and is familiar with the process of obtaining ACURO approval for animal research from the DoD. The EHA team also currently maintains IACUC protocols through Tufts University (via inter-institutional agreement) and will obtain IACUC approval through this mechanism for DEFUSE.



Bats are highly mobile and little is known of inter-cave migration/emigration rates. To **monitor bat roost fidelity and movement** we will mark *Rhinolophid* bats with individual Passive Integrated Transponder (PIT) tags to track individual bats' entry and exit from roost caves. Tags will be inserted subcutaneously between the bats' scapulae by trained personnel. The identities of individually tagged bats inhabiting roost caves will be recorded using radio frequency identification (RFID) data loggers and antennae at the roost entrances. Time-stamped data from individual bats collected by data loggers will be downloaded every 3 days to examine temporal roost site fidelity and rates of inter-cave immigration/emigration. Infrared video cameras will record the total number of bats flying out each night. Recapture data will be collected continuously throughout the project. We will attach radio transmitters (1.2g, Advanced Telemetry Systems, MN USA), to the back of 20 individual *Rhinolophus sinicus* and *Rhinolophus ferrumequinum* from each study roost (60 total) to determine nightly foraging patterns and local dispersal patterns. Telemetry data and PIT tag data will be used to calculate home range, to determine the degree of mixing among our three sites, and parameterize our dynamic models. We will use fine scale data on roost fidelity to determine the population mix at the specific roost sites (e.g. a side pocket of a cave where only one species roosts) for our

intervention. Radio transmitters that weigh <3% of bat body weight will be attached to the fur on the back using a veterinary dermatological adhesive (Vet Bond 3M, USA). We will collect location data from 60 bats (30 males, 30 females) every day for 10 days, 3 times per year for the 18 months of Phase 1. This will provide seasonal data to assess movement, including mating and gestation periods when higher levels of mixing and aggregation in the caves are expected.

High-risk SARSr-CoV quasispecies discovery, isolation and S. gene characterization. We will screen samples for SARSr-CoV nucleic acid using our pan-coronavirus consensus one-step hemi-nested RT-PCR (Invitrogen) assay targeting a 440-nt fragment in the RNA-dependent RNA polymerase gene (RdRp) of all known alpha- and betacoronaviruses assay[47,48], as well as specific assays for known SARSr-CoVs[27-30]. PCR products will be gel purified and sequenced with an ABI Prism 3730 DNA analyzer and quantitative PCR will be performed on SARSr-CoV-positive samples to determine viral load. Full-length genome of all detected SARSr-CoVs will be sequenced by high throughput sequencing method followed by genome walking. The sequencing libraries are constructed using NEBNext Ultra II DNA Library Prep Kit for Illumina and sequenced on a MiSeq sequencer, with PCR and Sanger sequencing used to fill gaps in the genome[29,30,32]. We will build phylogenetic trees using the Maximum Likelihood algorithm in the PhyML software, then scan for recombination events using Recombination Detection Program (RDP), confirmed using similarity plot and bootscan analyses in Simplot. We will analyze the S gene (which encodes the spike protein and determines receptor binding and cross-species transmission) of each sequence to identify a virus' potential to use human molecule ACE2 as a receptor. SARSr-CoVs with high similarity with SARS-CoV in full-length genomic sequences or with S proteins likely able to use human ACE2 as receptor will be identified as potential high-risk strains. We will then attempt isolation, cell culture, and infectious clone construction for further study *in vivo* and *in vitro* analysis. We have had success isolating and culturing SARSr-CoVs using Vero E6 monolayers in DMEM medium with 10% FCS, confirmed by RT-PCR and electron microscopy[29]. For SARSr-CoVs which we are not able to culture, we will construct recombinant viruses with the S gene of new bat SARSr-CoVs and the backbone of the infectious clone of SARSr-CoV WIV1 or of SARS-CoV, using the reverse genetic system described previously, and detailed below[28]. Initial assays of receptor usage and cell tropism will use various cell lines expressing human ACE2 incubated with isolated bat SARSr-CoVs or pseudotype viruses as previously shown[29].

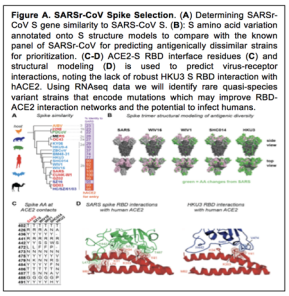
Approach to predicting bat SARSr-CoV spillover risk. Our approach is to combine state-of-the-art genotype-phenotype modeling with detailed step-wise experimental characterization of each bat SARSr-CoV we identify at our test cave sites.

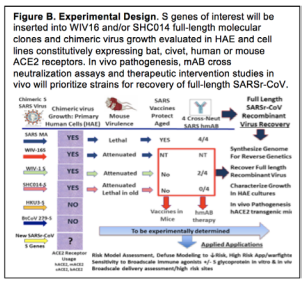
Flow chart here:

Sample testing/screening/Isolation – phylogenetic analysis/ACE2 binding modeling – ACE2

binding assays (all from Fig A) – chimera production – mouse model – SARS vaccines protect -cross neut humAB – full length recovery (all from Fig b)-) – Data into predictive modeling (additional box)

This flow chart should use some elements of Ralph's figures A and B as indicated. Ask Ralph to send you Figs A and B in editable format so you can fuse them in the way above (a chimera!), and without the text. The flow chart needs to have less detail so the flow is visible when shrunk down.





Our models will be parameterized with the experimental data from a series of assays on the S genes of bat SARSr-CoVs, with experimental and modeling work flowing together in iterative steps. The Baric laboratory pioneered many of the experimental approaches, the SARSr-CoV reverse genetic platforms, and full length S chimeric recombinant virus recovery from in silico sequence databases[7,8,23,49]. Full length recombinant strains reconstructed using reverse genetics in our lab include human epidemic strains, civet and raccoon dog SARS-CoV strains, and bat SARSr-CoVs (WIV16, WIV1, SHC014 and HKU3-SRBD repaired RBD interface). These strains will be used in the Baric, Shi and Wang laboratories for initial work on immune boosting and priming, and act as baseline data to parameterize the spillover risk modeling[7,8,23,49]. They will be supplemented by viruses we isolate under DEFUSE (worked on in the Shi lab) **and approximately 15-20 bat SARSr-CoV spike proteins/year** from DEFUSE (Baric, Shi labs). Most of the ~150 bat SARSr-CoV strains sequenced by us in prior work have not yet been examined for spillover potential and these will also be assessed in the following pipeline:

Experimental assays of SARSr-CoV spillover potential: *Ability to enter human cells:* Viral entry represents the key first step to evaluating the disease potential of SARSr-CoVs, with CoV species-specific restriction occurring primarily at entry[23,49]. To assess this we first will use structural modeling of SARSr-CoV S protein to ACE2 receptors. The structure of the SARS trimer prefusion S and the bound SARS-CoV S RBD to human and civet ACE2 have been solved, providing a platform for structural modeling and mapping hot spots of antigenic variation[50,51]. Mutations in the RBD[23,49,52,53], and host proteases and S glycoprotein proteolytic processing[54-56], regulate SARSr-CoV cell entry and cross-species infectivity. Mismatches in the S-RBD-ACE2 molecules or S proteolytic processing will prevent cell entry of SARS-CoV[23,49]. We will also

conduct *in vitro* pseudovirus binding assays, as we have done previously for WIV1 and others[29], as well as live virus binding assays for strains we are able to isolate. This work will be done in China (Shi lab), to prevent delays and unnecessary dissemination of viral cultures. *Novel SARSr-CoV Virus Recovery:* We will commercially synthesize select SARSr-CoV S glycoprotein genes, designed for insertion into our SHC014 or WIV16 molecular clone backbones (these viruses are 88% and 97% identical to epidemic SARS-Urbani in the S glycoprotein). These are BSL-3, not select agents, and pathogenic in hACE2 transgenic mice. Different backbone strains provide increased opportunities for recovery of viable viruses, and to identify potential barriers for RNA recombination-mediated gene transfer between strains[30]. Chimeric viruses will be recovered in Vero cells, or in mouse cells over-expressing human, bat or civet ACE2 receptors to support cultivation of viruses with a weaker RBD-human ACE2 interface. All chimeric viruses will be sequence verified and evaluated for: **i**) human, civet and bat ACE2 receptor usage *in vitro*, **ii**) growth in primary HAE, **iii**) sensitivity to broadly cross neutralizing human monoclonal antibodies (mAB) S215.17, S109.8, S227.14 and S230.15 and a mouse antibody (435) that recognize unique epitopes in the RBD[57,58] and **iv**) *in vivo* pathogenesis studies in hACE2 transgenic mice, using our well established approaches[7]. Should some isolates prove highly resistant to our mAB panel, we will evaluate cross neutralization against a limited number of human SARS-CoV serum samples from the Toronto outbreak in 2003 (n=10). Chimeric viruses that encode novel S genes with spillover potential (e.g. growth in HAE, use of multiple species ACE2 receptor for entry, antigenic variation) will be used to identify SARSr-CoV strains for recovery as full genome length viable viruses. *Recovery of Full length SARSr-CoV:* We will compile sequence/RNAseq data from a panel of closely related strains (e.g.<5% nucleotide variation) and compare the full length genomes, scanning for unique SNPs representing sequencing errors[59-61]. The genome of consensus candidates will be synthesized commercially (e.g. BioBasic), as six contiguous cDNA pieces linked by unique restriction endonuclease sites for full length genome assembly. Full length genomes will be transcribed into genome-length RNA and electroporation used to recover recombinant viruses[22,62]. We will re-evaluate virus growth in primary HAE cultures at low and high multiplicity of infections and *in vivo* in hACE2 transgenic mice, testing whether backbone genome sequence alters full length SARSr-CoV spillover potential. All experiments will be performed in triplicate and data provided to the Modeling Team in real time. We anticipate recovering ~3-5 full length genomes/yr, reflecting strain differences in antigenicity, receptor usage, growth in human cells and pathogenesis. *In vivo Pathogenesis:* We generated a mouse that expresses human ACE2 receptor under control of HFH4, a lung ciliated epithelial cell promoter[7]. Infection of this model with wildtype SARS-CoV results in lethal disease, but transient disease with bat SARSr-CoV WIV1, suggesting that WIV1 is less efficient at using hACE2 *in vivo* and less likely to produce severe disease in people initially on spillover. However, single amino acid variations in the SARS-CoV RBD of related strains could dramatically alter

these phenotypes, hence we will evaluate the impact of low abundant, high consequence micro-variation in the RBD. Groups of 10 animals will be infected intranasally with 1.0 x 10^4 PFU of each vSARSr-CoV, then clinical disease (weight loss, respiratory function by whole body plethysmography, mortality, etc.) followed for 6 days p.i.. Animals will be sacrificed at day 2 or 6 p.i. for virologic analysis, histopathology and immunohistochemistry of the lung and for 22-parameter complete blood count (CBC) and bronchiolar alveolar lavage (BAL) using the Vetscan HM5 (an instrument that measures parameters used for human clinical determination). *Identification of high risk/low abundant variants:* We will use RNAseq to identify low abundant quasispecies (QS) variants encoding mutations in RBD and/or residues that bind ACE2. These would alter risk assessment calculations as strains identified as low risk, might actually have low abundant, high risk variants circulating in the QS. To test this the Shi and Baric lab will structurally model and identify highly variable residue changes in the SARSr-CoV S RBD and use commercial gene blocks to introduce these changes singly and then in combination into the S glycoprotein gene of the low risk, highly abundant parental strain. We will examine the capacity of these low abundance chimeric viruses to use human, bat, civet and mouse ACE2 receptors, and to replicate in HAE cultures. *RBD deletions:* Small deletions at specific sites in the SARSr-CoV RBD leave the key RBD-ACE2 interface residues intact, such that Clade 1 strains represent higher risk of human infection (Fig. 5). We will analyze the functional consequences of these RBD deletions on SARSr-CoV hACE2 receptor usage, growth in HAE cultures and *in vivo* pathogenesis. First, we will delete these regions, sequentially and then in combination, in SHC014 and SARS-CoV Urbani, anticipating that the introduction of both deletions will prevent virus growth in Vero cells and HAE. We hypothesize that the smaller deletion may be tolerated, given its location in the RBD structure, so *in vivo* passage in the presence of receptor will restore growth, while identifying 2nd site reversions that restore efficient hACE2 usage[49]. In parallel, we will evaluate whether RBD deletion repair restores the ability of low risk strains to use human ACE2 and grow in human cells. To test this we will synthesize full length rs4237, a highly variable SARSr-CoV that encodes a few of the SHC014 RBD contact interface residues but also encodes a mutation at 479 (N479S) and has two deletions and hence, is not recoverable *in vitro*. Using the SHC014 backbone sequence, we will sequentially and then in tandem repair the deletions in the presence and absence of the S479N. We anticipate that the S479N mutation is critical given its key role in establishing the RBD-ACE2 interface, and that restoration of the RBD deletions will enhance virus recognition of hACE2

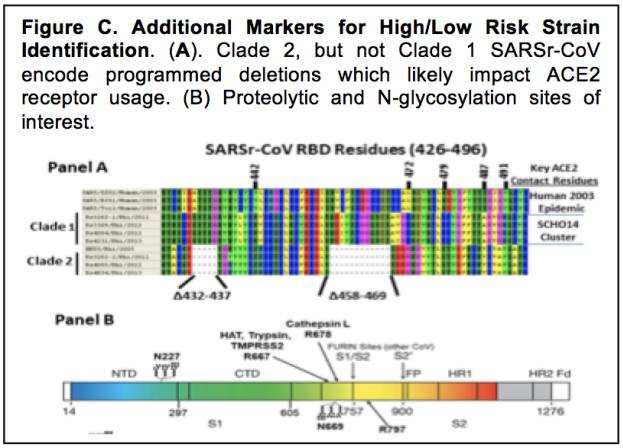


Figure C. Additional Markers for High/Low Risk Strain Identification. (**A**). Clade 2, but not Clade 1 SARSr-CoV encode programmed deletions which likely impact ACE2 receptor usage. (B) Proteolytic and N-glycosylation sites of interest.

receptors and growth in Vero cells and HAE cultures *S2 Proteolytic Cleave and Glycosylation Sites:* After receptor binding, a variety of cell surface or endosomal proteases[63-66] cleave the SARS-CoV S glycoprotein causing massive changes in S structure [67] and activating fusion-mediated entry[55], which is prevented in the absence of S cleavage[68] (Fig. 5). Tissue culture adaptations sometimes introduce a furin cleavage site which can direct entry processes, usually by cleaving S at positions 757 and 900 in S2 of other CoV, but not SARS[66]. For SARS-CoV, a variety of key cleavage sites in S have also been identified and we will analyze all SARSr-CoV S gene sequences for appropriately conserved proteolytic cleavage sites in S2 and for the presence of potential furin cleavage sites[69,70]. SARSr-CoV S with mismatches in proteolytic cleavage sites can be activated by exogenous trypsin or cathepsin L. Where clear mismatches occur, we will introduce the appropriate human-specific cleavage sites and evaluate growth potential in Vero cells and HAE cultures. In SARS-CoV, we will ablate several of these sites based on pseudotyped particle studies and evaluate the impact of select SARSr-CoV S changes on virus replication and pathogenesis (e.g. R667, R678, R797). We will also review deep sequence data for low abundant high risk SARSr-CoV that encode functional proteolytic cleavage sites, and if so, introduce these changes into the appropriate high abundant, low risk parental strain. *N-linked glycosylation:* SARS-CoV S has 23 potential N-linked glycosylation sites and 13 of these have been confirmed biochemically. Several of these regulate SARS-CoV particle binding DC-SIGN/L-SIGN, alternative entry receptors for SARS-CoV entry into macrophages/monocytes[71,72]. Mutations that introduced two new N-linked glycosylation sites may have been involved in the emergence of human SARS-CoV from civet and raccoon dogs[72]. While the sites are absent from civet and raccoon dog strains as well as clade 2 SARSr-CoV, they are present in WIV1, WIV16 and SHC014, supporting a potential role for these sites in host jumping. To evaluate this, we will sequentially introduce clade 2 residues at positions N227 and N699 of SARS-CoV and SHC014 and evaluate virus growth in Vero cells, nonpermissive cells ectopically expressing DC-SIGN and in HAE cultures, as well as in human monocytes and macrophages anticipating reduced virus growth efficiency. Using the clade 2 rs4237 molecular clone, we will introduce the clade I mutations that result in N-linked glycosylation sites at positions 227 and N699 and in rs4237 RBD deletion repaired strains, evaluating virus growth efficiency in HAE, Vero cells, or nonpermissive cells ± ectopic DC-SIGN expression[72]. *In vivo*, we will evaluate pathogenesis in transgenic ACE2 mice.

Models to predict viral spillover potential and evolution of high-risk SARSr-CoV strains. *Structural equation model of spillover potential:* We will use data from the experimental assays above to **build genotype-phenotype models of bat SARSr-CoV spillover potential**. We will use Bayesian Structural Equation Models (SEM), fit via MCMC methods[73], to predict spillover potential from the genetic traits of bat SARSr-CoVs and the ecological traits of hosts. SEMs have successfully analyzed the drivers of, and predicted stochastic species interactions[74,75]. They will

enable us to integrate multiple, interrelated tests of strain spillover potential into a common framework, while restricting relationships to plausible causal pathways. This prevents the over-fitting associated with a black-box approach. A Bayesian approach allows fitting with unbalanced and non-independent data, as per the larger number of cell-binding and cell-entry assays we will run to determine candidates for a smaller number of humanized mouse trials and LIPS assays (below). The viral traits derived from the experimental assays of spillover risk laid out above will be our primary set of predictor variables: presence of deletions in the RBD region, proteolytic binding sites, glycosylation sites, neutralization escape mutations, indeterminate mutations at high-variation sites found in low-abundance strains. We will include genetic similarity of each strain's RBD to the reference pandemic SARS-CoV genomes to test these aggregate measures as predictive proxies. To control for experimental conditions we will include whether assays were performed on live viral isolates, full-genome or synthetic chimeric viruses, and the molecular backbone used in the latter. These traits will be used as inputs to SEM's causal graph, and used to predict latent variables representing the interconnected processes that contribute to SARSr-CoV QS spillover potential: receptor binding, cell entry with and without the presence of exogenous proteases, immune system interaction, and intracellular growth, all measured by our laboratory assay. These, in turn will **act as predictors for the ultimate outcomes of host pathogenesis (Fig. 6)**. We will use previous work on these genetic traits to put informative priors on strength and direction of interactions in the causal graph. We will use **prior-knowledge model simulations to select target sequences from our sampling for characterization and genome-sequencing**, to collect data that maximally enhances the predictive power of our model. We will use regularizing priors to reduce over-fitting and help select the most predictive variables in the final predictive model.

Evolutionary modeling and simulation to predict potential strains: Our SEM modeling will generate estimates of the spillover potential of SARSr-CoV sequences from DEFUSE fieldwork and prior work. To examine risk associated with the total viral population at our test sites, we will model and simulate evolutionary processes **to identify likely viral QS that our sampling has not captured, as well as viral QS likely to arise in the future**. By estimating the spillover potential of these simulated QS, we can better characterize the risk associated with the total viral population. We will use a large dataset of S protein sequences and full-length genomes generated from prior work and DEFUSE fieldwork to estimate SARSr-CoV substitution rate and its genome-wide variation using coalescent and molecular clock models within a Bayesian MCMC framework[76]. We will then estimate SARSr-CoV recombination rates at the cave population level using the same dataset and Bayesian inference[77,78]. We will apply various methods (RDP[79], similarity plots, bootscan) to identify recombination breakpoints and hotspots within the SARSr-CoV genome. Using these estimates of substitution and recombination rates, we will simulate the evolution of the SARSr-CoV QS virome using a forward-time approach implemented in simulators that model specific RNA virus functions (e.g. VIRAPOPS[80]). This will

allow us to predict the rate at which new combinations of genetic traits can spread in viral populations and compare recombination rates among caves and bat communities. Our forward-simulated results **will provide a pool of likely unknown and future QS species**. Using these and our SEM model for spillover risk, **we will predict the QS that are most likely to arise *and* have pathogenetic and spillover potential.** We will use the evolutionary simulation results to iteratively improve our SEM model results. The number of genetic traits of interest for prediction of pathogenicity is potentially large, so we will perform variable reduction using tree-based clustering, treating highly co-occurring traits as joint clusters for purposes of prediction. We will generate these clusters from our full set of SARSr-COV sequences from DEFUSE fieldwork and prior work. However, as trait clusters may be modified in future virus evolution due to recombination, we will use our forward-evolutionary modeling to predict how well trait clusters will be conserved, retaining only those trait clusters unlikely to arise in unknown or future viral QS genomes. This will enable a good trade-off between increased predictive power based on current samples and generalizability to future strains that have not yet evolved.

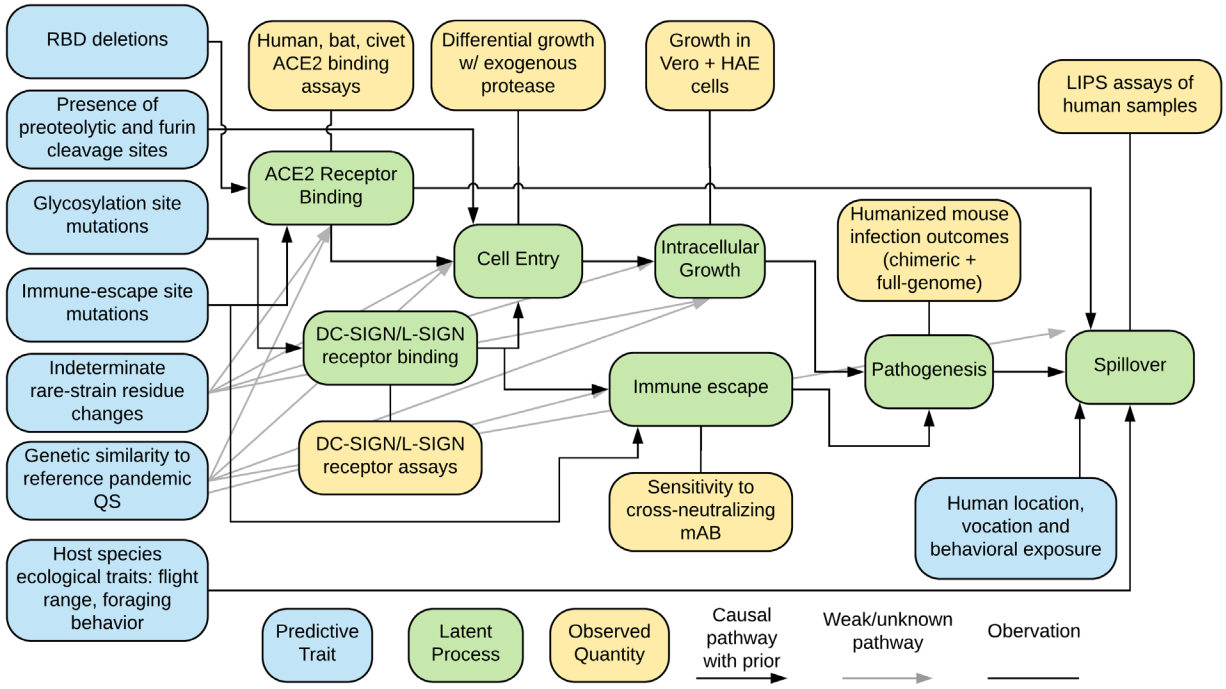


Figure 6: A simplified directed graph of a structural equation model representing the causal relationships between predictors and measures of viral pandemic potential.

Validation by LIPS assay on previously-collected human sera: Following our proof-of-concept field trial we will update these models to include not only pathogenesis but spillover probability validated with data on viral QS antibodies found in the local human population detected via Luciferase immunoprecipitation system (LIPS) assays on previously-collected human sera (NIAID project, Daszak PI). This includes >2,000 samples collected from people living close to our test

cave sites in Yunnan Province, and is the basis of a recent paper demonstrating 2.7% seropositivity to bat SARSr-CoVs in an initial sampling of this population[34] (Fig. 7). In addition to serum samples, extensive behavioral and wildlife contact data has been collected from this population, under an IRB that can be easily extended to cover DEFUSE work.

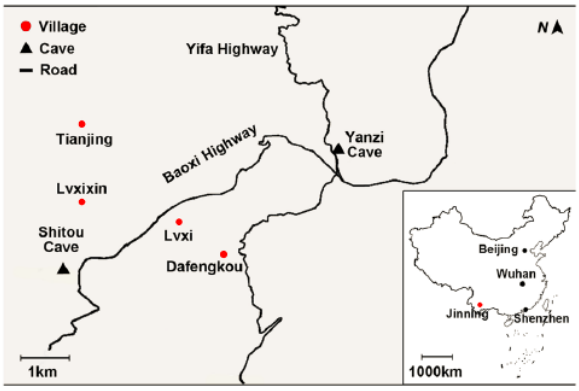


Fig. 7. Human sera were collection from villages (red dots) near bat caves where CoV positive samples have been isolated (Yanzi Cave and Shitou Cave, triangle).

Our ability to extend and validate these models with data on actual human contact and spillover allows us to fit and test models of actual, not just potential, spillover probability. Our previous work has shown that both host and viral traits predict zoonotic spillover from models[3], so in addition to viral traits, we will include key ecological traits of the host bat species in which viral QS were detected. These include flight ranges, foraging, roosting, demographic, and social behavior. To will use the extensive data on each person's behavioral exposure to wildlife, and their work, travel and occupational history, to correct for varying human exposure to bat species. We will design LIPS assays for specific high- and low-spillover risk SARSr-CoVs, to identify people who've been exposed to them, and test our model's validity. The LIPS uses viral antigens tagged with luciferase, from crude lysate, thereby eliminating the requirement for antigen purification and significantly reducing the time required for assay development and producing a more sensitive test than traditional ELISA[81]. Prof. Zhengli Shi (Wuhan Institute of Virology) will lead the LIPS serological work based on her 15 years SARSr-CoV human serological surveillance experience [82-84] and the recent success in SADS-CoV zoonotic risk study using LIPS[85]. To establish SARSr-CoV LIPS assays, we will: **1)** Insert different high- and low-risk SARSr-CoV N genes into pREN-2 vector (LIPS vector). We will first assess N gene similarity to determination their potential cross-reactivity in a LIPS assay. From our previous experience, SARSr-CoV maintain 80% similarity in the N protein, thus should be detectable using a universal SARSr-CoV N based LIPS assay; **2)** determine specificity of the LIPS assay by producing polyclonal sera via injection of recombinant protein or attenuated virus into rabbits. Selected SARSr-CoV N proteins or viral particles will be used as the immunogen for antibody production; **3)** validate SARS-CoV, MERS-CoV and SADS-CoV N protein LIPS assays by incubating antigens with their respective positive serum samples and the antigen antibody complex eluted using protein A/G beads. Luminescence is measured upon adding coelentrazine, a substrate of renilla luciferase. In a preliminary assay, LIPS successfully detected high strong antibody titer in the positive control serum sample, while the vector control did not show any response. Cut off was set as the average luminescence plus

three standard deviation from the control. We have used this to demonstrate efficacy for MERS-CoV and SADS-CoV (Fig. 8); **4)** validate LIPS positive sera results by spike protein based LIPS and viral neutralization assay. Similarly, S gene from high/low risk SARSr-CoV will be engineered into the pREN-2 vector and an S-LIPS assay produced, as above. As a confirmatory test the positive samples from LIPS, will be validated by viral neutralization assay. The data from LIPS and neutralization will be collected and analysis to validate the model.

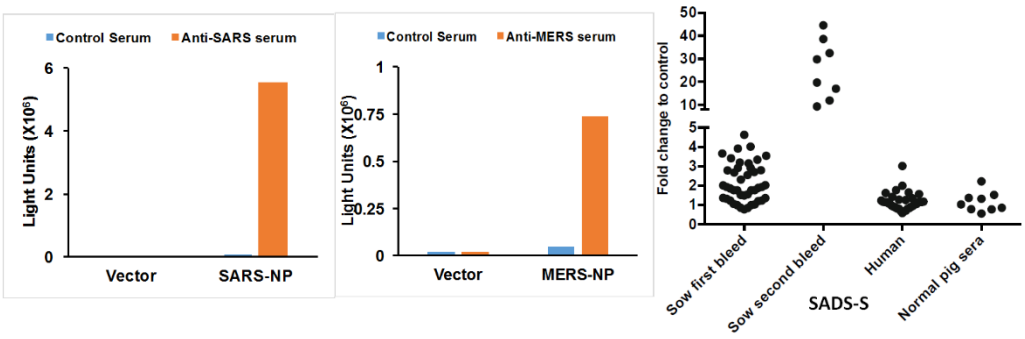


Fig. 8. LIPS assay was tested successful for SARS, MERS and SADS coronavirus N or S antibodies.

Thematic Area 2

Immune modulation approach to reducing bat SARSr-CoV spillover risk. There is no available technology to reduce the risk of exposure to novel CoVs from bats which carry zoonotic precursors to many emerging viruses including filoviruses (Ebola), CoV (SARS-CoV, MERS-CoV, etc.), paramyxoviruses (Nipah/Hendra), rhabdoviruses (rabies) and others. No vaccines or therapeutics exist for emerging CoVs, filoviruses and paramyxoviruses and exposure mitigation strategies are non-existent. We have shown that bats have unique immunological features that may explain why they coexist with viruses and rarely show clinical signs of infection. Our long-term studies demonstrate: a) bats maintain constitutively high expression of IFNα that may respond to and thus restrict, viral infection immediately[11]; b) several bat interferon activation pathways are dampened, e.g. STING (a central cytosolic DNA-sensor molecule to induce interferon) dependent and TLR7 dependent pathways[10]; c) the NLRP3 dependent inflammasome pathway is dampened, and some of the key inflammation response genes like AIM2 have been lost in bats[86,87]. The dampened IFN and inflammasome response suggest bats maintain a fine balance between IFN response and detrimental over-response. This is likely due to an adaptation of their immune-sensing pathways as a fitness cost of flight[9]. We hypothesize that the bat innate/adaptive immune responses are quite different from that of human and mouse. Firstly, virus replication will likely be restricted quickly by constitutively expressed IFNα in bats, resulting in lower B/T cell stimulation due to lower viral stimuli. Second, dampened interferon and inflammasome responses will result in lower cytokine responses that are

required to trigger T/B cell dependent adaptive immunity (e.g. antibody response). The strong innate immune response, due to the lack of an efficient antibody response, will clear the virus. **We and others have demonstrated proof-of-concept of this phenomenon:** Experimental Marburg virus infection of Egyptian fruits bats, a natural reservoir host, resulted in wide tissue distribution yet low to moderate viral loads, brief viremia, low seroconversion and a low antibody titer that waned quickly, suggesting no long-term protection is established[88-90]. Similarly, poor neutralizing antibody responses occur after experimental infection of bats with Tacaribe virus[91] and in our studies with SARS-CoV experimentally infected bats (L-F Wang, unpublished data). Indeed, we successfully showed bat interferon can inhibit bat SARSr-CoVs[28]. We hypothesize that if we can use immune modulators that upregulate the naturally low innate immunity of bats to their viruses, we will be able to transiently suppress viral replication and shedding, reducing the risk of spillover. We will evaluate two immune modulation approaches to defuse spillover of SARSr-CoVs from bats to humans: **1) Broadscale Immune Boosting strategies (Wang, Duke-NUS):** we will apply immune modulators like TLR-ligands, small molecule Rig like receptor (RLR) agonists or bat interferon in live bats, to up-regulate their innate immunity and assess suppression of viral replication and shedding; **2) Targeted Immune Priming (Baric, UNC):** the broadscale immune boosting approach will be applied in the presence and absence of chimeric immunogens to boost clearance of high-risk SARSr-CoVs. Building on preliminary development of polyvalent chimeric recombinant SARSr-CoV spike proteins, we will use novel chimeric polyvalent recombinant S proteins in microparticle encapsidated gels and powders for oral delivery and/or virus adjuvanted immune boosting strategies where chimeric recombinant SARSr-CoV S are expressed from raccoon poxvirus, which has been used extensively to deliver rabies immunogens in bats and other animals. We will conduct application trials with live bats to assess suppression of replication and shedding of a broad range of pathogenic SARS-related CoVs. Both lines of work will begin in Year 1 and run parallel, be assessed competitively for efficiency, cost, and scalability, and successful candidates used in our live bat trials at our test sites in Yunnan, China. We believe an immune boosting/priming strategy is a superior approach for this challenge because solutions are likely to be broadly applicable to many bat species, and across many viral families.

Broadscale immune boosting (led by Wang, Duke-NUS). We will work on the following key leads to identify the most effective approach to up-regulate innate immunity an suppress viral loads. *Toll-like receptor (TLR)/Rig-I Like Receptor (RLR) ligands:* We have begun profiling bat innate immune activation *in vivo*, in response to various stimuli. Our work indicates a robust response to TLR-stimuli like polyI:C when delivered *in vivo*, as measured by transcriptomics on spleen tissue (Fig. 7). We have performed transcriptomics on spleen, liver, lung and lymph node, with matched proteomics to characterize immune activation *in vivo*. These activation profiles will be used to assess the bat immune response to different stimuli and direct the

response to favor those which lower the viral load in our experimental system at Duke-NUS (below). In addition to the ligands already tested, we will stimulate the Rig-I pathway with 5'pppDSRNA, a mimetic of the natural RIG-I stimulant. These stimulants will activate functional bat IFN production pathways, and a similar strategy has been demonstrated in a mouse model for clearance of SARS-CoV, influenza A virus and Hepatitis B virus[12,15].

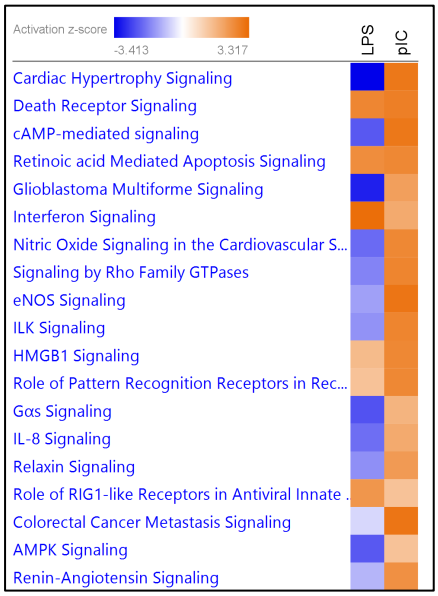


Fig. 7. Pathway analyses from Ingenuity Pathway Analysis (IPA) of whole spleen NGS after stimulation with either LPS or polyI:C. Z-score increase over control bats is indicated as per scale, and suggests strong activation of many pathways.

Universal bat interferon: To overcome any complications arising from species-specificity, we will design a conserved universal bat interferon protein sequence and produce purified protein. Utilization of a universal IFN for bats will overcome species-dependent response to the ligand, allowing the use of IFN throughout broad geographical and ecological environments and across many bat species. As a starting point, we have produced recombinant non-universal, tagged, bat IFN that are effective at inducing appropriate immune activation (Fig. 8). This ligand can be **delivered by aerosol or intranasal application** as has been shown to reduce viral titers in humans, ferrets and mouse models[12,13,15]. Interferon has been used clinically in humans as an effective countermeasure when antiviral drugs are unavailable, e.g. against filoviruses[14]. Replication of SARSr-CoV is sensitive to IFN treatments, as shown in our previous work[28]. The successful delivery, immune activation and outcome on the host will be characterized thoroughly to optimize rapid immune activation.

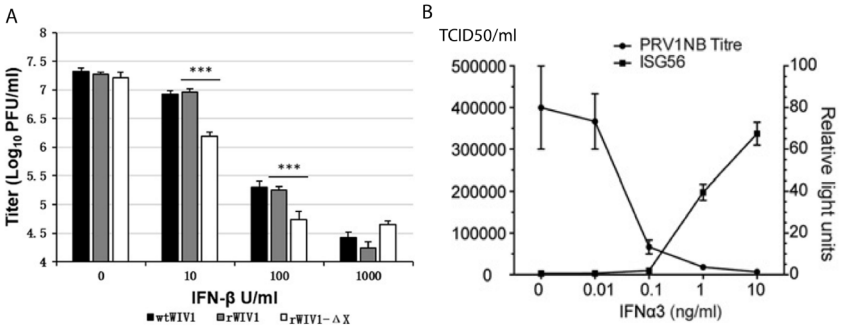


Fig. 8: Bat viruses are sensitive to IFN treatments. A) Recombinant bat SARS-related coronavirus WIV1 replication was inhibited by human IFN-β in a dose dependent manner in Vero cells. B) Bat reovirus PRV1NB replication was inhibited by recombinant bat IFNα3 in a dose dependent manner in bat PakiT03 cells.

Boosting bat IFN by blocking bat-specific IFN negative regulators: Uniquely, bat IFNα is naturally constitutively expressed but cannot be induced to a high level, indicating a negative regulatory factor in the bat interferon production pathway[92]. To fast-track the identification of this target

we will utilize a *Pteropus alecto* CRISPRi library pool that we have created covering multiple RNA targets in every gene in the *P. alecto* genome. The library has already been produced and genes affecting influenza replication in bat cells have been identified. Using CRISPRi we can identify negative regulator genes and then screen for compounds targeting these genes to boost the inducibility of the IFN system in a shorter time-frame. Based on previous work, it is highly likely this will be a conserved pathway throughout the order *Chiroptera*. *Activating dampened bat-specific innate immune pathways which include DNA-STING-dependent and TLR-dependent pathways:* Our work showing that mutant bat STING or reconstitution of AIM2 and functional NLRP3 homologs restores antiviral functionality suggests these pathways are important in bat-viral coexistence and that the majority of the pathway is preserved. By identifying small molecules to directly activate pathways downstream of STING or TLR/RLRs, such as TBK1 activation, we will activate bat innate defense by interferons and promote viral clearance. We hypothesize that these small molecules we will be able to significantly reduce viral load in bats. *Validation in a bat-mouse model.* Various CoVs show efficient infection and replication inside the human host but exhibit defective entry and replication using mouse as a host due in part to differences in DPP3 and ACE2 receptors. We have shown efficient reconstitution of irradiated mice using bat bone marrow from multiple species, including *E. spelaea*. Fig. 9 shows the efficient reconstitution of bat PBMC's in the mouse, presence of circulating bat cells and generation of bat-specific antibodies in mice incapable of producing an antibody response. This 'batized' mouse model can be utilized for both circulating infection of SARS/MERS CoV (in the immune compartment only) and as a model for generating bat-specific antibodies against CoV proteins. Efficient validation of infection into bat cells will be used to validate the infectivity of the viruses and generation of bat antibodies will facilitate validation of the best proteins/peptide to elicit an effective immune response.

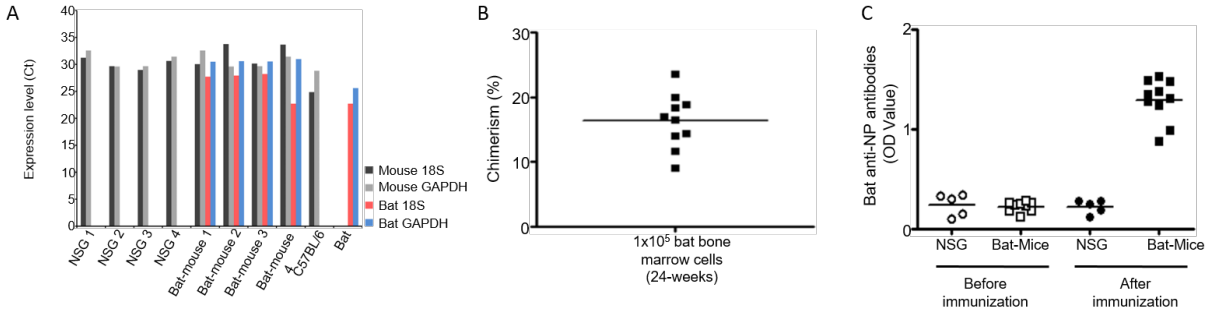


Fig. 9: A) Presence of bat-specific qPCR in reconstituted mice after 12 weeks. B) chimeric ratio of bat-mouse cells in circulation after 24 weeks. C) Specific antibody response to a KLH-tetanus antigen generated by bat-reconstituted mice.

Viral infection models in cave-nectar bat (Duke-NUS): To test and compare the efficacy of the immune modulating approaches above, we will use our cave-nectar bat (*Eonycteris spelaea*)

breeding colony infected with Melaka virus (family *Reoviridae*) which is known to infect this species[93,94]. We will also use two coronaviruses (SARSr-CoV WIV1 and MERS-CoV in ABSL3. Details of infection, housing, prior infection trials in the facility… Viral loads will be measured by qPCR, titration of produced virus, NGS transcriptomics and nanostring probes added to the immunoprofiling panel. Antibody responses will be measured by LIPS assay. This approach allows us to test our immune-boosting strategies, in a safe and controlled environment, prior to expanding to field-based evaluation. The analytical methods used for the *E. spelaea* colony will be replicated to analyze the experimental infection of *Rhinolophus* in a wild-cave scenario. Additionally, the versatility of the analysis should allow easy application to multiple species of bats

Targeted Immune Priming (led by Baric, UNC). We have developed novel group 2b SARSr-CoV chimeric S glycoproteins that encode neutralizing domains from phylogenetically distant strains (e.g. Urbani, HKU3, BtCoV 279), which differ by ~25%. The chimeric S programs efficient expression when introduced in the HKU3 backbone full length genome, and elicit protective immunity against multiple group 2b strains. We will develop robust expression systems for SARSr-CoV chimeric S using ectopic expression *in vitro*. Then, we will work with Dr. Ainslie (UNC-Pharmacy) who has developed **novel microparticle delivery systems and dry powders for aerosol release**, and which encapsidate recombinant proteins and adjuvants (innate immune agonists) that will be used for **parallel broadscale immune boosting strategies ± chimeric immungens.** Simultaneously, we will introduce chimeric and wildtype S in raccoon poxvirus (RCN), in collaboration with Dr. Rocke and confirm recombinant protein expression, first *in vitro* and then in the Duke-NUS bat colony, prior to any field trial. The goal of this aim is to develop a suite of reagents to remotely reduce exposure risk in high risk environmental settings.

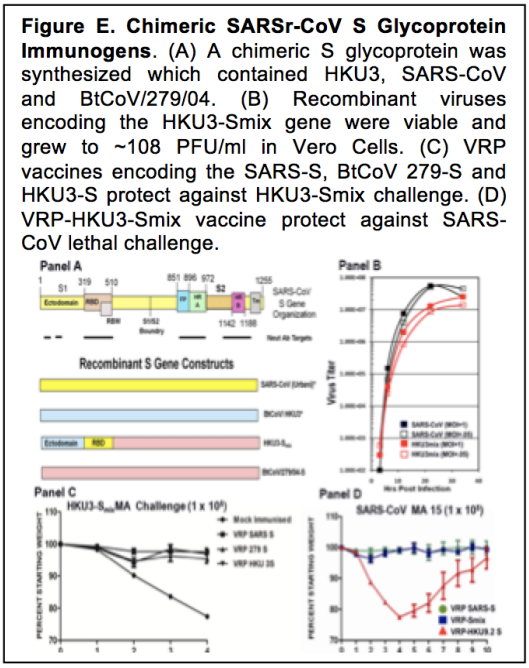


Figure E. Chimeric SARSr-CoV S Glycoprotein Immunogens. (A) A chimeric S glycoprotein was synthesized which contained HKU3, SARS-CoV and BtCoV/279/04. (B) Recombinant viruses encoding the HKU3-Smix gene were viable and grew to ~108 PFU/ml in Vero Cells. (C) VRP vaccines encoding the SARS-S, BtCoV 279-S and HKU3-S protect against HKU3-Smix challenge. (D) VRP-HKU3-Smix vaccine protect against SARS-CoV lethal challenge.

Chimeric SARSr-CoV S Immunogens: CoV evolve quickly by mutation and RNA recombination, the latter provides a strategy to rapidly exchange functional motifs within the S glycoprotein and generate viruses with novel properties in terms of host range and pathogenesis[30,95]. CoV also encode neutralizing epitopes in the amino terminal domain (NTD), RBD and S2 portion of the S glycoprotein[57,96,97], providing a strategy to build chimeric immunogens that induce broadly cross reactive neutralizing antibodies. Given the breadth of SARSr-CoV circulating in

natural settings, chimeric immunogens will be designed to increase the breadth of neutralizing epitopes across the group 2b phylogenetic subgroup[40]. Using synthetic genomes and structure guided design, we fused the NTD of HKU3 (1-319) with the SARS-CoV RBD (320-510) with the remaining BtCoV 279/04 S glycoprotein molecule (511-1255), introduced the chimeric S glycoprotein gene into the HKU3 genome backbone (25% different than SARS-CoV, clade 2 virus) and recovered viable viruses (HKU3-S_{mix}) that could replicate to titers of about 10^8 PFU/ml on Vero cells (Fig. 10). HKU3-Smix is fully neutralized by mAb that specifically target the SARS RBD (data not shown). In parallel, we inserted the $HKU3_{mix}$ S glycoprotein gene into VEE virus replicon vectors (VRP-$S_{chimera}$) and demonstrated that VRP vaccines protect against lethal SARS-CoV challenge and virus growth. In addition, VRP-S_{HKU3} and VRP-S_{279} both protect against $HKU3_{mix}$ challenge and growth *in vivo* (Fig. 9), demonstrating that neutralizing epitopes in the $HKU3_{mix}$ S glycoprotein are appropriately presented and provide broad cross protection against multiple SARSr-CoV strains. In addition to using these immunogens as a targeted broad-based boosting strategy in bats, we will also produce a chimeric SHC014/SARS-CoV/HKU3 S and a SCH014/SARS-CoV/WIV-1 S gene for more focused immune targeting on known high risk strains. In parallel, we will work with the Protein Expression Core at UNC (https://www.med.unc.edu/csb/pep) to produce codon optimized, stabilized and purified prefusion SARS-CoV glycoprotein ectodomains as published previously[17]. Purified recombinant protein will be used by Drs. Rocke and Ainslie for inclusion in delivery matrices (e.g. purified powders, dextran beads, gels – see below) with broadscale immune agonists (adjuvants-Dr. Wang) like poly IC, TLR4 and Sting agonists.

2nd Generation Chimeric S glycoprotein Design and Testing: We will also produce a chimeric SHC014 NTD/SARS-CoV-RBD/HKU3 S C terminal and generate recombinant HKU3 encoding the trimer spike (HKU3-S_{S014}), for more focused immune targeting on known high and low risk strains designated from our experimental and modeling analyses. A second construct will be synthesized with a SHC014 NTD domain, SARS-CoV RBD and WIV-1 C terminal domain (WIV-S_{S014}). After sequence variation, we will evaluate virus growth in Vero and HAE cultures and the ability of SARS RBD monoclonal antibodies (S227, S230, S109) to neutralize chimeric virus infectivity[89,96]. We will also evaluate *in vivo* pathogenesis in C57BL/6 mice and hACE2 transgenic mice. The recombinant HKU3-S_{S014} S genes will be introduced into VRP vectors and sent to Dr. Rocke for insertion into the raccoon poxvirus vaccine vector. Using established techniques, we will characterize S expression and then provide virus vectors to Prof. Wang for immune boosting trials at Duke-NUS, and ultimately if successful in the field (Prof. Shi). We will also synthesize human codon optimized the HKU3-S_{S014}, WIV-S_{S014} and HKU3-S_{mix} chimeric spikes for expression and purification by the UNC proteomics core, producing mg quantities for inclusion in nanoparticle and microparticle carriers in collaboration with Dr. Ainslie. We will produce enough material for *in vivo* testing in mice and in bats. Recombinant HKU3-S_{S014} and WIV-S_{S014} glycoprotein expression will be validated by Western blot and by vaccination of mice, allowing

us to determine if the recombinant protein elicits neutralizing antibodies that protect against lethal SARS-CoV, HKU3-S_{mix} and SHC014 challenge. In parallel, we will survey the RNAseq data for evidence of complex S glycoprotein gene RNA recombinants in the SARSr-CoV population genetic structure. If present, we will synthesize 2-3 interesting recombinant S genes, insert these genes into SHCO14 or HKU3 genome backbones and VRP and characterize the viability and replicative properties of these viruses in cell culture and in mice and the VRP for S glycoprotein expression and vaccine outcomes. We will produce immunogens and evaluate their ability to protect against infection.

Adjuvant and Immunogen Delivery Vehicles. Dr. Ainslie (UNC) and collaborators have developed the biodegradable polymer acetalated dextran (Ac-DEX) for the delivery of antigens and adjuvants in vaccine applications (Fig. 11). Ac-DEX has distinct advantages over other polymers for vaccine development: 1) synthesis is straightforward and scalable. An FDA-approved water soluble dextran polysaccharide is modified and rendered insoluble in water by a simple one-step modification of its hydroxyl groups with pendant acyclic or cyclic acetal groups[98-100]. Unlike other dextran based vaccine materials, our material is acid sensitive, which has been shown to greatly improve antigen presentation; 2) Ac-DEX microparticles (MPs) can passively target antigen-presenting cells (APCs) based on their size (5-8µm), being phagocytosed by DCs and traffic to the lymph node[101]. Furthermore, APCs have acidic phagosomes that can result in triggered intracellular release due to the acid-sensitivity of Ace-DEX; 3) Ac-DEX MPs and their hydrolytic byproducts are pH-neutral, biocompatible, and safe compared to other commonly used polyesters have acidic hydrolytic byproducts (e.g. lactic and glycolic acid, in the case of PLGA) that damage vaccine components such as protein antigens[102]. The complete hydrolysis of Ac-DEX results in particle breakdown with release of the metabolic side products. 4) Ac-DEX MPs are stable outside the cold-chain. MPs can be stored for at least 3 months at 45°C without any loss of integrity or encapsulated cargo bioactivity[103]. Other common formulations (e.g. liposomes[104], PLGA MPs[103], squalene emulsions [Fluad™ package insert]) have limited shelf-life that requires the cold-chain. Ac-DEX MPs can be aerosolized, or delivered in sprays or gels to bat populations, providing new modalities for zoonotic virus disease control in wildlife populations[98,105]. 5) We have previously encapsulated Poly (I:C)(1), resiquimod[101], and a STING agonist into our novel MPs[106].

As seen in Fig. 10, encapsulation of Poly (I:C) drastically enhances the activity of the TLR agonist. Additionally, encapsulation of adjuvants in MPs drastically enhances the activity of subunit vaccines. We have

Figure F. Particle Delivery Systems. Broadscale immune boosting strategies include (A) Dextran microparticles or Dry nanoparticle powders. (B) Macrophages cultured with either free poly (I:C) or poly (I:C) encapsulated into Ac-DEX MPs produce significant TNFα. (C) Comparison of (left) neutralizing titer and (right) viral load when ferrets are vaccinated with Ac-DEX MPs. Day 0, 28, and 56 (prime, boost, and challenge.)

displayed better efficacy than state-of-the-art FDA-approved inactivated flu virus (Fluarix) in a ferret model of influenza. The ferret model is the ideal animal model for influenza because of their relatively small size and they possess various clinical features associated with human influenza infection[107]. This formulation used HA with encapsulated STING agonist cyclic [G(3',5')pA(3',5')p](16)

Microparticle Performance Metrics in vitro and in Rodents and Bats: MPs are designed for aerosol delivery due to their relatively effective low aerodynamic diameter[108], their low density microporous nature which allows for efficient aerosol dispersal and deep penetration into the lung, or deposition on the skin for oral uptake by grooming. We will encapsulate Poly (I:C), resiquimod (TLR 7) or other innate immune agonists to enhance type I interferon production in in consultation with Prof Wang. Agonist laden particles will be made separately or in combination with recombinant SARS-CoV chimeric spike proteins, encapsulated into our aerodynamic MPs as well as nanoparticles.

Delivery system development (Rocke, NWHC). We have previously developed, tested and registered oral vaccines and delivery methods to manage disease in free-ranging wildlife including a sylvatic plague vaccine for prairie dogs[24], vaccines against bat rabies[25] and white-nose syndrome (unpubl. data). We have optimized vaccine delivery methods, uptake by the target species and safety in non-target hosts using biomarkers prior to deployment[109]. We will use a similar approach to develop, test and optimize delivery methods to *Rhinolophus* bats in SE Asia. While work on immune modulating agents progresses, we will concurrently develop and test mediums, routes, and methods of delivery to large colonies of bats. We will determine the most feasible and simple method of delivery that achieves high uptake by bats, is safe for humans as well as target and non-target species, and minimizes colony disturbance. Sticky edible gels or pastes that bats groom from themselves and each other have been used previously to deliver pharmaceuticals to bats orally and we are currently testing these for use in rabies vaccine delivery. These may also be useful for delivering immune modulators and recombinant SARSr-CoV spike proteins to *Rhinolophus* bats, but may need to be combined with viral vectors (like poxvirus or adenovirus) or nanoparticles/nanoemulsions that enhance uptake through mucous membranes or transdermally after topical application. *Poxvirus vectors:* Poxviruses are effective viral vectors for delivering vaccines to wildlife [24,110,111], and can replicate safely at high levels in bats after oronasal administration[26]. We have demonstrated proof-of-concept in bats. We modified vaccinia Ankara (MVA) and raccoon poxvirus (RCN) vecotrs for safety and replication in bats using *in vivo* biophotonic imaging[25]. RCN replicated to higher levels in bats than MVA, even via the oral route, and was found to be highly safe for bats (Fig. 12). We used raccoon poxvirus-vectored novel rabies glycoprotein (mosaic or MoG) and demonstrated protective efficacy in bats after oronasal and topical administration[25] (Fig. 13). We are currently developing vaccine delivery for vampire bats in several Latin American

countries, and vaccines for white-nose syndrome in bats, a devastating disease that has killed millions of insectivorous bats in North America.

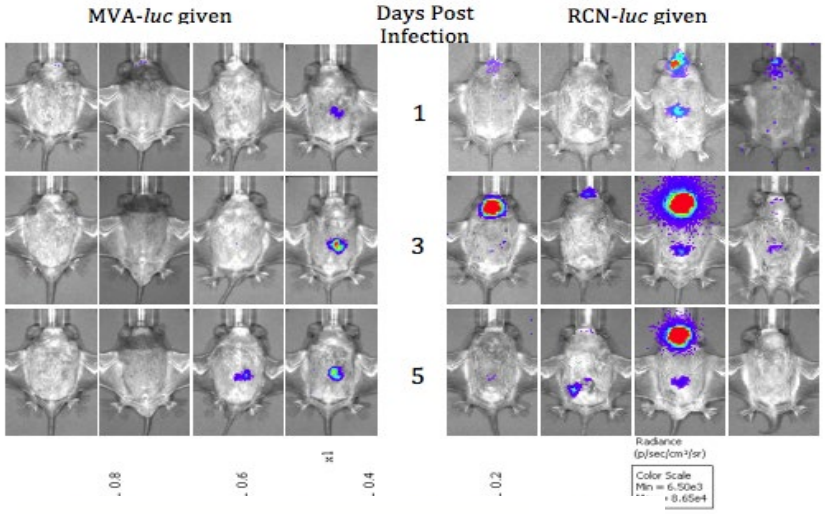


Fig. 12. Luminescence, indicative of viral replication of modified vaccinia Ankara (MVA) and raccoon poxvirus RCN) in the bat Tadarida brasiliensis *on 1, 3 and 5 dpi via the oronasal route.*

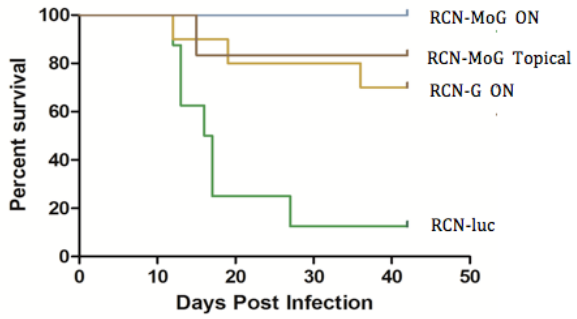


Figure 13. Vaccine efficacy and rabies challenge in Epstesicus fuscus immunized with raccoon poxvirus expressing a mosaic G protein (RCN-MoG) either oronasally (ON) or topically in comparison to RCN expressing typical G protein and RCN expressing luciferase (negative control).

Poxviruses are safe in a wide variety of wild and domestic animals, and allow for large inserts of foreign DNA. We have previously used a raccoon poxvirus vectored vaccine expressing plague antigens was incorporated into a peanut-butter flavored bait matrix to manage plague caused by *Yersinia pestis* in prairie dogs. We incorporated the biomarker Rhodamine B (RB) into baits to assess uptake by target and non-target species [109,112] (Fig. 14). RB is visible under a UV microscope until the hair grows out (~50 days in prairie dogs). We have since conducted a large field trial (approved by USDA Center for Veterinary Biologics) that demonstrated vaccine efficacy in four species of prairie dogs in seven western states[24]. We used biomarker analysis to assess site- and individual host-specific factors that increased bait consumption including age, weight, and the availability of green vegetation.

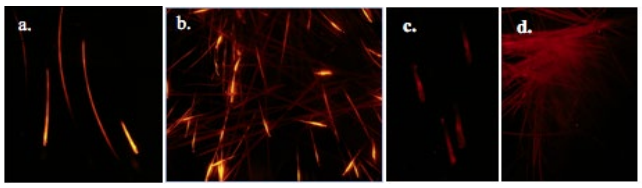


Fig. 14. Prairie dog hair and whisker samples under fluorescence microscope (excitation wavelength: 540 nm, emission wavelength: 625 nm) to determine uptake of baits containing Rhodamine B. a) 20 days after

bait distribution, b) 16 days after bait distribution, c) and d) controls (note natural dull fluorescence).

Transcutaneous delivery: In addition to viral, we will also consider methods to achieve transcutaneous delivery of the immune boosting proteins without the use of live agents. Nanoparticles have been used to increase transcutaneous delivery efficiency[113]. However, the impermeable stratum corneum provides a difficult barrier to breach. Mechanical approaches have been used[113] but are somewhat unethical and impractical for wildlife. We are currently testing poly lactic-co-glycolic acid (PLGA) as a nanoparticle to encapsulate rabies glycoprotein as a method of transcutaneous delivery of vaccine to bats via dendritic cell uptake[114], as has been shown for delivery of TLR agonists and antigens simultaneously to mice[115]. This approach will be competitively trialed against ac-DEX to encapsulate and deliver SARSr-CoV glycoproteins, with and without adjuvants[116], e.g. Matrix M1 (Isconova, Sweden) which has been shown to significantly enhance the immune response in mice to SARS-CoV spike proteins[18]. For efficiency and to reduce costs, initial trials will be conducted in the USA with locally acquired insectivorous big brown bats (*Eptesicus fuscus*) which we have maintained and housed for several experiments at our facility previously[25,26]. We will treat bats via topical application with various test formulations that include the biomarker Rhodamine B (RB), co-house them with untreated bats, and monitor transfer between bats by collecting hair and whiskers for biomarker analysis.

Initial field trials: Bat are not attracted to baits, so delivery in the field is challenging. The high rates of self and mutual grooming observed in bats has previously been exploited to cull vampire bats using poisons like warfarin, applied topically to a small number of bats. Once released, contact and mutual grooming transfers the poison within the colony. We have conducted preliminary biomarker studies in vampire bats in both Mexico and Peru and also in insectivorous bats in Wisconsin. In Peru, we conducted trials with RB-labeled glycerin jelly. Based on capture-recapture data, we estimated a rate of transfer from 1.3 – 2.8 bats for every bat marked. We are analyzing factors associated with rates of transfer, e.g. sex and age of initially treated bats, time of day, to model the rate of vaccination and impact on rabies transmission with different rates of application, prior to actual deployment of vaccine in the field. More recently, we applied RB marked glycerin jelly to the entry of bat houses used by little brown bats (*Myotis lucifugus*). Of 29 bats trapped one week post-application, 59% were positive for biomarker indicating they had eaten the jelly. We will conduct initial trials with each of the delivery vehicles in caves in Wisconsin, targeting local US insectivorous bats. Within one week of application, bats will be trapped at the cave entrance using mist nets or Harp traps and hair will be collected to assess the rate of uptake via biomarker analysis. The bats will be released immediately afterward. The procedures will be tested at several different locations as it will likely take some manipulation to determine appropriate dosages for maximum uptake. After we have determined the most optimal approaches for mass delivery, we will then test

them on wild bats in our three cave sites in Yunnan Province. Again, biomarker will be used to assess rates of uptake and this data can then be used in modeling studies to help determine the optimal rates of application of immunomodulating agents. Biomarker studies can also be used to assess uptake by non-target species, an important consideration in evaluating safety. Fieldwork will be conducted in collaboration with Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance).

Innovative Aerosol Approach to Bat Inoculation: Once we have confirmed uptake in laboratory studies, we will then assess scalable delivery methods in local caves and hibernacula (using biomarker-labeled mediums but without immunomodulatory substances). In collaboration with Dr. Jerome Unidad of Palo Alto Research Center (PARC), we will develop an innovative aerosol platform technology unique to PARC into a field-deployable prototype for use in cave settings. The technology called Filament Extension Atomization (FEA) can spray fluids with a wide-range of viscosities ranging from 1mPa-s (the viscosity of saliva and most aqueous vaccine formulations) up to 600Pa-s (the viscosity of creams and gels for topical delivery) using a roll-to-roll misting process (https://www.parc.com/services/focus-area/amds/) that results in narrowly-dispersed droplets with tunable sizes from 5-500 microns. FEA technology is compatible with all the formulations of interest to project DEFUSE, including aqueous formulations intended for conventional spraying and the edible gels and creams intended for topical delivery with no limit on bioactive ingredient loading. FEA can then be a universal delivery platform for direct spraying onto bats with the formulation geared towards bio-efficacy.

We will subcontract to PARC to develop a field-deployable FEA prototype, potential form factors for which are shown in Fig. 15F, that can be used in cave settings. PARC will develop the prototype in close collaboration with USGS-NWHC and will conduct the initial trials with them on Wisconsin cave bats. After initial trials, PARC will develop the prototype to a form that will be used for the proof-of-concept demonstration at the test sites in the Kunming bat caves, Yunnan province, China. The field-deployable system will be motion-actuated, and on a timer so that bats will be targeted at fly-in and fly-out but diurnal flying non-target species (e.g. cave swiftlets) can be avoided.

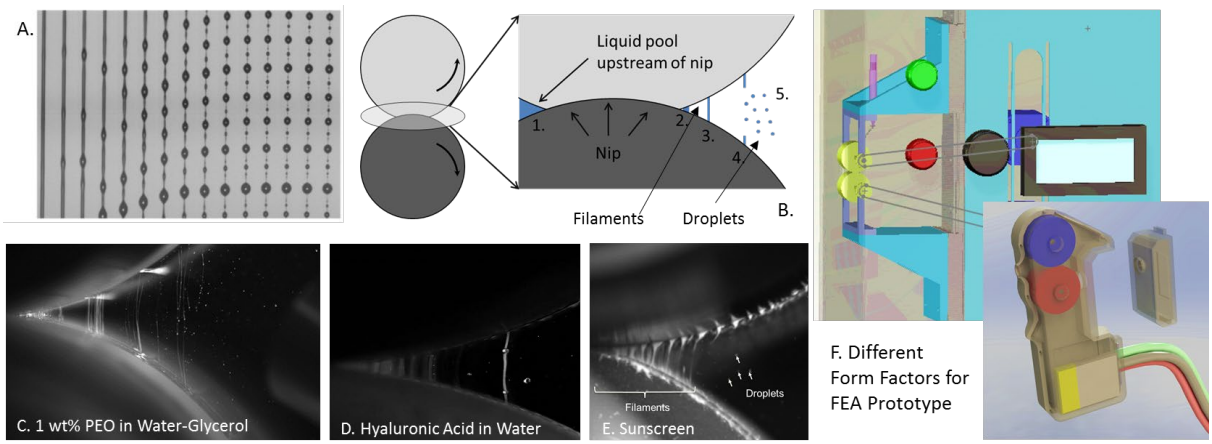


Fig. 15: PARC FEA Technology – A. Beads-on-a-string structures in viscoelastic fluids, B. Parallelization of filament formation and droplet break-up in an FEA roller system, C.-E. Images from high speed videos of representative fluids sprayed with FEA (Polyethylene Oxide in Water-Glycerol, Hyaluronic Acid and Sunscreen), F. Potential form factors of the field-deployable prototype for Project DEFUSE (benchtop, handheld)

Dynamic circulation modeling to optimize deployment strategy. To select amongst various options for immune boosting, priming, and targeting, and multiple delivery options and schedules, we will simulate deployment using a model of viral circulation in cave bat populations. The model will be fit to data from our three-cave test system but designed to be robust to be generalizable to other cases. We will simulate outcomes under a variety of different deployment scenarios to produce conservative estimate of necessary application under real-world conditions. *Fit stochastic viral circulation models to longitudinal sampling data:* We will use longitudinal viral prevalence, mark-recapture estimates of bat populations, radiotelemetry and infrared camera data collected during our field sampling to parameterize and construct models of bat population dynamics and viral circulation in our test caves. We will use a simple but robust stochastic SIR process model with immigration and emigration and flexible, nonlinear contact rates between bats[117]. This model structure can capture a wide range of viral dynamics from intermittent viral outbreaks to regular, endemic circulation with a relatively small number of parameters. We will fit these models to our sampling data using the **partially observable markov process (pomp)** framework[118], allowing estimates of the underlying latent dynamic disease transmission process, accounting for and separating the natural stochasticity of viral circulation and observation error in sampling. We will validate our models via temporal cross-validation: leaving out successive sections on longitudinal time-series from our model fitting to test the model, and by testing the results of a fit from two cave sites on data from a third. *Simulate circulation under a set of plausible deployment scenarios.* Using the top performing sets of immune boosting and targeted immune priming molecules from captive trials, and the delivery media and methods with the greatest uptake rates in cave

studies, we will use the stochastic SIR model to generate simulations of viral circulation under a series of treatment deployments in our focal study caves. These scenarios will cover a range of plausible intensities, frequencies, and combinations of suppression strategies. They will incorporate uncertainty in the efficacy of each of the treatment strategies. From these simulations, we will estimate the expected degree and time period of suppression of viral circulation and shedding and the uncertainty in this expectations. We will determine the optimal scenario for deployment in our focal study caves. *Test robustness of deployment strategies under broader conditions:* We will use our simulation models to determine best strategies for deployment under a variety of conditions covering likely environments. We anticipate the deployment is likely to occur under (a) highly varied species population and compositions, with uncertain estimates based on rough observations (b) varied uptake and efficacy of immune boosting and targeting molecules due to different environmental conditions, and (c) limited time or resources to deploy treatment. Thus, we will simulate deployment under many potential conditions to determine how optimal deployment differs according to condition, and determine deployment strategies which are conservative and robust to these uncertainties and limitations.

Proof-of-concept deployment of immune modulation molecules in test caves in Yunnan Province, China.

MANAGEMENT PLAN

- Provide a summary of expertise of the team, including any subcontractors, and key personnel who will be doing the work. Resumes count against the page count.

- Identify a principal investigator for the project.

- Provide a clear description of the team's organization

- Include an organization chart with the following information, as applicable:
  A) Programmatic relationship of team members
  B) Unique capabilities of team members
  C) Task responsibilities of team members
  D) Teaming strategy among the team members
  E) Key personnel with amount of effort to be expended by each during each year

- Provide a detailed plan for coordination including explicit guidelines for interaction among

collaborators/subcontractors of the proposed effort.

- Include risk management approaches.

- Describe any formal teaming agreements that are required to execute this program.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research organization focused on emerging zoonotic diseases. The PI, Dr. Peter Daszak, has 25+ years' experience managing lab, field and modeling research projects on emerging zoonoses. Dr. Daszak will commit 3 months annually to oversee and coordinate all project activities, and lead modeling and analytic work for TA1. Dr. Billy Karesh has 40+ years' experience leading zoonotic and wildlife disease projects, and will commit 1 month annually to manage partnership activities and outreach. Dr. Jon Epstein, with 15 years' experience working emerging bat zoonoses will coordinate animal trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project. Support staff include field surveillance teams, modeling analysts, and consultants based in Yunnan Province, China, to oversee field trials. The EHA team has worked extensively with all other collaborators: Prof. Wang (15+ years); Dr. Shi (15+ years); Prof. Baric (5+ years) and Dr. Rocke (15+ years). **Subcontracts: #1** to Prof. Ralph Baric, UNC, to oversee reverse engineering of SARSr-CoVs, BSL-3 humanized mouse experimental infections, design and testing of immune priming treatments based on recombinant spike proteins. Assisted by senior personnel Dr. Tim Sheahan, Dr. Amy Sims, and support staff; **#2** to Prof. Linfa Wang, Duke NUS, to oversee the immune boosting approach, captive bat experiments, and analyze immunological and virological responses to immune boosting treatments; **#3** to Dr. Zhengli Shi, Wuhan Institute of Virology, to conduct PCR testing, viral discovery and isolation from bat samples collected in China, spike protein binding assays, and some humanized mouse work, as well as experimental trials on *Rhinolophus* bats. Her team will include Dr. Peng Zhou and support staff; **#4** to Dr. Tonie Rocke, USGS National Wildlife Health Center, to refine delivery mechanisms for both immune boosting and immune priming treatments. With a research technician, Dr. Rocke will use a captive colony of bats at NWHC for initial trials, and oversee cave experiments in China; **#5** to Dr. Jerome Unidad, PARC, to develop their innovative aerosol platform into a field-deployable device for large-scale inoculation of the bats. Dr. Unidad will collaborate closely with Dr. Rocke in developing a field-deployable prototype for both initial trials and cave experiments in China.

Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based research organization focused on emerging zoonotic diseases. His >300 scientific papers include the first global map of EID hotspots[119,120], estimates of unknown viral diversity[121], predictive models of

virus-host relationships[3], and evidence of the bat origin of SARS-CoV[29] and other emerging viruses [122-125]. He is Chair of the NASEM Forum on Microbial Threats, and is a member of the Executive Committee and the EHA institutional lead for the $130 million USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr. Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Dept. of Epidemiology and Dept. of Microbiology & Immunology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, cross species transmission and pathogenesis. His group has developed a platform strategy to access the potential "pre-epidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (REFS).

Prof. Linfa Wang is Director, Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore. His proven track record in the field includes identifying the bat origin of SARS-CoV, pioneering work on Henipaviruses and many more. His work has shifted from identifying the bat-origin of pathogens to understanding basic bat biology and the mechanisms by which they can endure sustained virus infection. He has received multiple awards including the 2014 Eureka Prize for Research in Infectious Diseases. He currently heads and administers a Singapore National Research Foundation grant on "Learning from bats" for $9.7M SGD. He is an advisory member of …. an Editor of multiple journals and current Editor-in-Chief for the Journal *Virology*.

Dr. Danielle Anderson is the Scientific Director of the Duke-NUS ABSL3 laboratory and is an expert in RNA virus replication. Dr Anderson has extensive experience in both molecular biology and animal models and will lead the animal studies. Dr Anderson has established Zika, Influenza and Reovirus non-human primate (NHP) models in Singapore, using different inoculation routes (such as mosquito inoculation), and has performed trials on over 30 NHPs.

Dr Aaron Irving is an experienced postdoctoral fellow in the field of innate immunity and viral sensing with expertise focusing on host-pathogen interactions and intrinsic immunity. He oversees multiple projects on bat immune activation within Prof. Linfa Wang's laboratory at Duke-NUS Medical School and has experience in *in vivo* animal infection models.

Prof. Zhengli Shi: Dr. Shi is the director of the Center for Emerging Infectious Diseases of the Wuhan Institute of Virology, Chinese Academy of Sciences. She got Ph.D training in Virology in Montpellier University II from 1996 to 2000, biosafety training at Australian Animal Health Laboratory in May 2006 and at Lyon P4 in October 2006. She is now in charge of the scientific

activity in BSL3 and BSL4 of the Institute. Her research focuses on viral pathogen discovery through traditional and high-throughput sequencing techniques. She has been studying the wildlife-borne viral pathogens, particularly bat-borne viruses since 2004. Her group has discovered diverse novel viruses/virus antibodies in bats, included SARS-like coronaviruses, adenoviruses, adeno-associated viruses, circoviruses, paramyxoviruses and filoviruses in China. One of her great contributions is to uncover genetically diverse SARS-like coronaviruses in bats with her international collaborators and provide unequivocal evidence that bats are natural reservoir of SARS-CoV by isolation of one strain that is closely related to the SARS-CoV in 2002-3. She has coauthored >100 publications on viral pathogen identification, diagnosis and epidemiology.

Dr. Tonie Rocke is a

Dr. Jerome Unidad is a Member of Research Staff at the Hardware Systems Laboratory at PARC. His research interests revolve around novel fluid delivery systems (including aerosol delivery) for high viscosity fluids, polymers and biomacromolecules. At PARC, he is the technical lead in developing the FEA spray technology for consumer and biomedical applications, as well as additive manufacturing. He has a PhD in Chemical Engineering, specializing in polymer science and rheology, from the University of Naples "Federico II" in Naples, Italy and was a postdoctoral researcher at Forschungszentrum Juelich in Munich, Germany.

Dr. Peng Zhou is a
Dr. Xinglou Yang
Dr. Ben Hu

Dr. Kevin Olival is VP for Research at EcoHealth Alliance. His research over the last 15 years has focused on understanding the ecology and evolution of emerging zoonoses, with a focus on developing analytical tools and modeling approaches to forecast and prioritize the discovery and surveillance of viral zoonoses. This includes a recent large scale analysis identifying host and viral predictors of spillover in mammals [REF, Nature]. He has led several international field teams to investigate bat-borne viruses globally. Dr. Olival is the Modeling and Analytics coordinator for the USAID PREDICT-2 project; co-PI on an NIH-NIAID project to investigate CoVs in China; and PI on recent DTRA-CBEP grant to characterize CoVs from bats in Western Asia.

Please follow the same format and create Bios for all other personnel with Ph.D and higher. Peter Daszak will then work out how much space we have and decide who to include…

CAPABILITIES

- Describe organizational experience in relevant subject area(s), existing intellectual property, specialized facilities, and any Government-furnished materials or information.

- Discuss any work in closely related research areas and previous accomplishments.

(The following information was taken from the 'Goals and Impact' section of the abstract we submitted).

The SARSr-CoV-bat system, and immune modulation focus: Our group's 15 yrs work on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014 & WIV-1). We have shown they bind and replicate efficiently in primary human lung airway cells and that chimeras with SARSr-CoV spike proteins in a SARS-CoV backbone cause SARS-like illness in humanized mice, with clinical signs that are not reduced by SARS monoclonal therapy or vaccination. We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV *(7,8,9)*. We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**. Our work on bat immunology suggests that bats' unique flying ability has led to downregulated innate immune genes, and their ability to coexist with viruses such as SARSr-CoVs, henipa- and filoviruses that are lethal in many other mammals *(3)*. We have identified bat-specific constitutively expressed bat interferon, a dampened STING-interferon production pathway *(4, 5)*, and have identified a series of other innate immunity factors that are dampened in bats *(6)*.

STATEMENT OF WORK

- Provide a detailed task breakdown, citing specific tasks and their connection to the interim milestones and program metrics.

- Each phase of the program (Phase I base and Phase II option) should be separately defined in the SOW and each task should be identified by TA (1 or 2).

NOTE: The SOW must not include proprietary information.

- For each task/subtask, provide:
    - A detailed description of the approach to be taken to accomplish each defined task/subtask.
    - Identification of the primary organization responsible for task execution (prime contractor, subcontractor(s), consultant(s), by name).
    - A measurable milestone, i.e., a deliverable, demonstration, or other event/activity that marks task completion. Include quantitative metrics.
    - A definition of all deliverables (e.g. data, reports, software) to be provided to the Government in support of the proposed tasks/subtasks.

Phase I:

TA-01 Task 1.1 Construct species distribution models to predict viral spillover risk in cave bats in South and Southeast Asia
Sub-task 1.1.1.;lkj;lkj;klj
Sub-task 1.1.2.;lj;lkj;lkj
Deliverables: models capable of …..

TA-01 Task 2.5: Field studies to collect tolerant reservoir species. (EcoHealth Alliance, William Karesh).

Sub-Task 2.5.1. Apply for and obtain IACUC approval and appropriate wildlife permits in Bangladesh for sample collection. Collection of blood and urogenital, oropharyngeal and rectal swab specimens from targeted bat, rodent and non-human primate species from Bangladesh (n = 1000 specimens). Collection of wing-punch dermal tissue biopsies from bats (n = 300).

Sub-Task 2.5.2. Field work is to be conducted by a trained field team using ethical, nondestructive capture, restraint, and sample collection techniques (with IACUC and local government approval). Samples are to be preserved in RNA later (or other preservative) to maintain cellular integrity and frozen at the point of collection using a liquid nitrogen dry shipper and maintained in -80^{o}C. All samples are to be shipped with appropriate government permission and export permits.

Deliverables: 1000 field specimens (whole blood, nasal/rectal swabs) collected from reservoir bats, rodents and non-human primates which have been obtained with all proper permits and permissions are appropriately shipped for further analysis.

TA1:
Task 1.1
Sub-task 1.1.1. Models to predict bat community in caves across S. and SE Asia.

Organization leading task: EcoHealth Alliance
Sub-task 1.1.2. Models to predict presence of viruses with zoonotic potential in bats across S. and SE Asia.

Progress Metrics:

- Joint species distribution model fit for Asian Bats
- Cave-level predictions of bat community composition
- Linear predictions of viral diversity in cave populations
- JSDM predictions of viral diversity in cave populations
- Prediction validations

Deliverable(s):

- Deployable spatial model software of bat community composition
- Deployable spatial model software of viral diversity in bat cave populations

Progress Metrics:

- Joint species distribution model fit for Asian Bats
- Cave-level predictions of bat community composition
- Linear predictions of viral diversity in cave populations
- JSDM predictions of viral diversity in cave populations
- Prediction validations

Deliverable(s):

- Deployable spatial model software of bat community composition
- Deployable spatial model software of viral diversity in bat cave populations

Subtask 1.1.3: Develop prototype app for the warfighter
Description and execution:
Preliminary Data:
Organization leading task: EcoHealth Alliance
Progress Metrics: Development of fully functional and user-friendly application. Use of application in the field.
Deliverables:

Task 1.2: Determining baseline risk of SARSr-CoV emergence in Yunnan, China
Subtask 1.2.1. Longitudinal sampling of bats to determine virus prevalence and diversity in Yunnan cave sites.

Subtask 1.2.2. Analyzing ability of CoVs to infect and emerge in people

***(TA1)* Subtask 5: Assay SARr-CoV quasispecies for spillover potential via assays for binding, cell entry, and pathogenesis in mouse models.**

Organization leading task: University of North Carolina

Progress Metrics: Not sure how to do this.

Deliverable(s):

1. *Methods to Produce Synthetic SARSr-CoV Virus Molecular Clones and Reverse Genetics.*
    a. ***Preliminary Data**:* Molecular Clones for SARSr-CoV WIV1, WIV16, SHC014 and HKU3-SRBD exist. We have demonstrated in the preliminary data that these reagents are already available.
    b. ***Target Goals**:* We will generate molecular constructs for 20+ chimeric SARSr-CoV encoding different S glycoprotein genes/yr
    c. **Target Goals***: We will generate 2-5 full length molecular clones of SARSr-CoV.*

2. *Methods of Recombinant virus Recovery and Characterization*
    a. **Preliminary Data***: Demonstrated recovery recombinant chimeric SARSr-CoV WIV1, WIV16, SHC014, HKU3-SRBD, including full length recombinant viruses of WIV1, WIV16, SHC014 and HKU3-SRBD.*
    b. *Target Goals:* We will isolate 20+ chimeric SARSr-CoV encoding novel S glycoprotein genes
    c. *Target Goals:* We will isolate 2-5 full length SARSr-CoV/year/
        i. **Key Deliverables for Program-wide Success***: These two key reagents position us for immediate testing of the antiviral effects of* **broadscale immune boosting molecules** +/- immunogens on virus growth *in vitro* and *in vivo*, and on virus levels in models of chronic SARS-CoV infection in mice.

3. *Virus Phenotyping: Receptor Interactions and In Vitro Growth.*
    a. ***Preliminary Data**: Cell lines encoding bat, human, civet and mouse ACE2 receptors exist and have been validated. We have demonstrated the use of primary human airway epithelial cultures to characterize SARSr-CoV pre-epidemic potential.*

b. ***Target Goals****: We will characterize SARSr-CoV recombinant virus growth in Vero cells, nonpermissive cells encoding the civet, bat and human ACE2 receptors.*

4. *Virus Pathogenic Potential in Humans:*
    a. *Preliminary Data: We also have transgenic human ACE2 mouse models to compare the pathogenic potential of SARSr-CoV*
    b. *Target Goals: We will evaluate SARSr-CoV pathogenic outcomes in hACE2 transgenic mice.*

5. *Virus Antigenic Variation:*
    a. *Preliminary Data: We have robust panels of broadly cross reactive human monoclonal antibodies against SARS and related viruses and mouse models to evaluate protection against SARSr-CoV replication and pathogenesis.*
    b. *We will evaluate SARS-vaccine performance against a select subset of SARSr-CoV (10), chosen based on the overall percent of antigenic variation, coupled with distribution across the S glycoprotein structure.*

6. *Low Abundant High Consequence Sequence Variants:*
    a. *We will identify the presence of low abundant, high risk SARSr-CoV, based on deep sequencing data*

7. *Proteolytic Processing and Pre-epidemic Potential.*
    a. *We will evaluate the role of proteolytic cleavage site variation on SARSr-CoV cross species transmission and pathogenesis in vivo.*

(TA1) **Subtask 4: Build models to predict viral species spillover potential and evoluation**

Organization leading task: EcoHealth Alliance

Description and execution:

Progress Metrics:

- *Development of prior-based pathogenicity predictions and sequence testing guidance*
- *Model fits from initial rounds of viral characterization*
- *Model fits from secondary rounds of viral characterization*
- *Predictions of spillover probability of sequenced viral QS*
- *Deployable predictive model*

Deliverable(s):

- Fit models as reproducible, deployable software providing virus spillover potential predictions and uncertainties based on input of host species and viral sequence data
- Ranking of potential pathogenicity of virus QS from both Task X sampling and previous data.

***(TA2)* Task 5: Trial experimental approaches aimed towards 'Broadscale Immune Boosting' using experimental bat colonies**

<u>TA2:</u> Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

Organization leading task: Wuhan Institute of Virology, Duke-NUS

***(TA2)* Task 6: Trial experimental approaches aimed towards 'Immune Targeting' using experimental bat colonies**

Organization leading task: University of North Carolina

Progress Metrics:

Deliverable(s):

1. ***Chimeric S-Glycoprotein Antigen Design, Recovery and Phenotyping for Immune Boosting***.
    a. **Preliminary Data***: Demonstrated recovery recombinant chimeric HKU3-S_{mix}, demonstrating preservation of entry functions in the chimeric spike. Neutralizing epitopes and in vivo pathogenesis phenotypes were also preserved. Chimeric Spikes are biologically functional.*
    b. ***Target Goals**:* We will isolate chimeric HKU3-S_{S014} S and WIV-S_{S014} genes, chimeric viruses and express the S glycoprotein from VRP and raccoon poxvirus expression vectors.
    c. ***Target Goals:*** We will synthesize 2-3 chimeric S glycoproteins, recover recombinant viruses derived from natural recombinants in the population genetic structure of SARSr-CoV. We will also characterized recombinant protein expression from VRP and raccoon poxviruses.
    d. ***Target Goals**:* We produce sufficient recombinant HKU3-S_{S014}, WIV-S_{S014} and HKU3-S_{mix} S glycoproteins for inclusion in nanoparticle and microparticle delivery vehicles.
        i. **Key Deliverables for Program-wide Success***: These two key reagents*

position us for immediate testing of the antiviral effects of **broadscale immune boosting molecules** +/- immunogens.

2. ***Virus Phenotyping: Receptor Interactions and Growth in vitro and in vivo****.*
    a. ***Preliminary Data****: We have well developed metrics for evaluating chimeric S glycoprotein function in the context of whole virus, neutralization phenotypes and expression as recombinant proteins vaccines for testing in mice.*
    b. ***Target Goals****: Demonstrate chimeric S function in the context of virus infection in Vero and HAE cells and susceptibility to neutralizing antibodies targeted the SARS RBD.*
    c. ***Target Goals****: Evaluate chimeric virus pathogenesis in hACE2 transgenic mice and the ability of VRP vaccines encoding chimeric spikes to elicit protective immunity against lethal SARS-CoV, HKU3-S_{mix} and SCH014 challenge.*

3. ***Production of Agonist (TLR4, dsRNA, Sting) and Chimeric S glycoprotein Nanoparticle and Microparticle Suspensions for in vivo studies***
    a. ***Preliminary Data****: Robust preliminary data exists on the production and immunogenicity of nanoparticle and microparticle delivery systems.*
    b. ***Target Goals****: Produce nanoparticle and microparticle delivery systems encoding agonists, coupled with in vitro testing in vitro in bat and in other reporter cells, mice and bats.*
    c. ***Target Goals****: Inclusion of chimeric recombinant proteins and agonists in nanoparticle and microparticle delivery vehicles, coupled with testing in vitro and in vivo in mice and bats.*
    d. ***Target Goals***: Perform *in vivo* testing in collaboration with Dr. Shi and Dr. Wang.

SCHEDULE AND MILESTONES

- Provide a detailed schedule showing tasks (task name, duration, work breakdown structure element as applicable, performing organization), milestones, and the interrelationships among tasks.

  NOTE: Task structure must be consistent with that in the SOW.

- Measurable milestones should be clearly articulated and defined in time relative to the start of the project.

PREEMPT TRANSITION PLAN

- Indicate the types of partners (e.g. government, private industry, non-profit)

- Submit a timeline with incremental milestones toward successful engagement.
  NOTE: begin transition activities during the early stages of the program (Phase I).

- Describe any potential DARPA roles.

Project DEFUSE partners come from academic, government, private industry, private non-profit institutions and will develop a coherent transition plan for research findings, data and any technology developed in this work.

PARC as a private industry partner (large business) is a fully-owned subsidiary of Xerox Corporation and is committed to commercializing the FEA technology through IP licensing for different applications spaces to different commercial partners. In the context of project DEFUSE, PARC has been and will continue to engage potential licensees (OEMs) in the biotechnology and biomedical fields for eventual transitioning of targeted delivery technology that might result in the project. PARC already has existing networks of business relations in the biotechnology and biomedical space, both large companies (Fortune 500, Fortune 1000) and small businesses and start-ups who could be transition partners for FEA as a wide-scale, large-area drug delivery device. In addition, in collaboration with our extended network of DEFUSE partners and with DARPA, we will further identify existing government needs for our delivery technology, particularly in wildlife health management (in collaboration with EHA and USGS-NWHC) as well as in suppression of emerging threats (in collaboration with government agencies such as the CDC). PARC will leverage this knowledge in developing a needs-based commercialization plan with potential partners.

PREEMPT RISK MITIGATION PLAN

- Provide the following:

    - An assessment of potential risks to public health, agriculture, plants, animals, the environment, and national security.

    - Guidelines the proposer will follow to ensure maximal biosafety and biosecurity.

    - A communication plan that addresses content, timing, and the extent of distribution of potentially sensitive dual-use information. The plan must also address how input from DARPA, other government, and community stakeholders

will be taken into account in decisions regarding communication and publication of potentially sensitive dual-use information.

ETHICAL, LEGAL, SOCIETAL IMPLICATIONS

- Address potential ethical, legal, and societal implications of the proposed technology.

BIBLIOGRAPHY

A) Brief Bibliography (no page limit indicated – can be published/unpublished)
This and next part don't count toward 36 page limit

RELEVANT PAPERS

B) Up to 3 relevant papers attached (optional)
Propose:

- Ge et al. Nature
- Menacherry et al.
- Zhou et al. SADS-CoV

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[EXTERNAL] Important - response needed FW: PREEMPT - Language on 'Fundamental Research'

Peter Daszak <daszak@ecohealthalliance.org>

Sun 3/25/2018 10:46 AM

To: Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>; Rocke, Tonie E <trocke@usgs.gov>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>

Ralph, Linfa, Jerome, Tonie,

Please read the email below from Luke. We need to know if any of your part of the proposed work for DEFUSE will be considered 'proprietary' or 'restricted'. The definitions are in the attached doc, as per the email below. Those of you who've had experience with DoD funding will likely know the answer for this, but please reply to all, so that the others can follow your lead on this.

We do need responses ASAP, so we can get the correct language into the proposal.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4474
www.ecohealthalliance.org
@PeterDaszak
@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Luke Hamel [mailto:hamel@ecohealthalliance.org]
Sent: Saturday, March 24, 2018 5:24 PM
To: Peter Daszak; William B. Karesh; Jon Epstein; Kevin Olival, PhD
Subject: PREEMPT - Language on 'Fundamental Research'

Hi all,

Within our proposal, **we must mention whether we believe the scope of the research included in our proposal is 'fundamental' or not.**

I've attached a document with language from the BAA (pp. 17,18) that defines 'fundamental' research and distinguishes it from 'proprietary/restricted' research.

We've discussed this topic before, but I'm not sure we ever reached a consensus on how we're defining our work. **If we believe some of our collaborators' research may be 'proprietary', this is something we need to discuss with them immediately, as this is something we must address in the proposal.**

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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2.2. FUNDAMENTAL RESEARCH

It is DoD policy that the publication of products of fundamental research will remain unrestricted to the maximum extent possible. National Security Decision Directive (NSDD) 189 defines fundamental research as follows:

> 'Fundamental research' means basic and applied research in science and engineering, the results of which ordinarily are published and shared broadly within the scientific community, as distinguished from proprietary research and from industrial development, design, production, and product utilization, the results of which ordinarily are restricted for proprietary or national security reasons.

As of the date of publication of this BAA, the Government expects that program goals as described herein may be met by proposers intending to perform fundamental research and proposers not intending to perform fundamental research or the proposed research may present a high likelihood of disclosing performance characteristics of military systems or manufacturing technologies that are unique and critical to defense. Based on the nature of the performer and the nature of the work, the Government anticipates that some awards will include restrictions on the resultant research that will require the awardee to seek DARPA permission before publishing any information or results relative to the program.

Proposers should indicate in their proposal whether they believe the scope of the research included in their proposal is fundamental or not. While proposers should clearly explain the intended results of their research, the Government shall have sole discretion to select award instrument type and to negotiate all instrument terms and conditions with selectees. Appropriate clauses will be included in resultant awards for non-fundamental research to prescribe publication requirements and other restrictions, as appropriate. This clause can be found at http://www.darpa.mil/work-with-us/additional-baa.

For certain research projects, it may be possible that although the research being performed by the awardee is restricted research, a subawardee may be conducting fundamental research. In those cases, it is the awardee's responsibility to explain in their proposal why its subawardee's effort is fundamental research

[EXTERNAL] PREEMPT - Facility information

Luke Hamel <hamel@ecohealthalliance.org>

Sun 3/25/2018 3:55 PM

To: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>
Cc: Rocke, Tonie E <trocke@usgs.gov>

Hi Rachel and Katie,

Within the 'Capabilities' section of the PREEMPT proposal, we must include language about each of our collaborator's facilities. I apologize if I've already requested this information from you, but in the event that I haven't, could you please write-up a short paragraph for this section? I've attached a template to this email for you to follow.

Please return this section to me as soon as you are able.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

*(Please use the text below as a template for describing your research facility).

Rocky Mountain Laboratories (RML); Hamilton, MT — The Integrated Research Facility, which opened on the RML campus in 2008, houses BSL-4 laboratory space for conducting research on viral agents requiring maximum containment. The BSL-4 lab is equipped with all items needed to perform classic virology, basic immunology, and molecular biology, and the space is equipped for handling caged animals including rodents, nonhuman primates, and small livestock animals such as pigs, goats, and sheep. The Virus Ecology Unit (led by Dr. Munster) has field sites in Africa and the Middle East to study the role of fruit bats in the ecology of EBOV and is developing a bat colony on site at RML.

[EXTERNAL] RE: Important - response needed FW: PREEMPT - Language on 'Fundamental Research'

Wang Linfa <linfa.wang@duke-nus.edu.sg>

Sun 3/25/2018 4:37 PM

To: Peter Daszak <daszak@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>; Rocke, Tonie E <trocke@usgs.gov>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>

Dear Peter,

At this stage, I don't think we will have 'proprietary' or 'restricted' research as most of our work is published or to be published soon.

Thanks

LF

Linfa (Lin-Fa) WANG, PhD FTSE
Professor & Director
Programme in Emerging Infectious Disease
Duke-NUS Medical School,
8 College Road, Singapore 169857
Tel: +65 6516 8397

From: Peter Daszak [mailto:daszak@ecohealthalliance.org]
Sent: Sunday, 25 March, 2018 11:46 PM
To: Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Jerome.Unidad@parc.com; Rocke, Tonie
Cc: William B. Karesh; Luke Hamel; Anna Willoughby
Subject: Important - response needed FW: PREEMPT - Language on 'Fundamental Research'
Importance: High

Ralph, Linfa, Jerome, Tonie,

Please read the email below from Luke. We need to know if any of your part of the proposed work for DEFUSE will be considered 'proprietary' or 'restricted'. The definitions are in the attached doc, as per the email below. Those of you who've had experience with DoD funding will likely know the answer for this, but please reply to all, so that the others can follow your lead on this.

We do need responses ASAP, so we can get the correct language into the proposal.

Cheers,

Peter

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President

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Sent: Saturday, March 24, 2018 5:24 PM
To: Peter Daszak; William B. Karesh; Jon Epstein; Kevin Olival, PhD
Subject: PREEMPT - Language on 'Fundamental Research'

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I've attached a document with language from the BAA (pp. 17,18) that defines 'fundamental' research and distinguishes it from 'proprietary/restricted' research.

We've discussed this topic before, but I'm not sure we ever reached a consensus on how we're defining our work. **If we believe some of our collaborators' research may be 'proprietary', this is something we need to discuss with them immediately, as this is something we must address in the proposal.**

Best,

Luke Hamel
Program Assistant

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---

intended recipient, please delete it and notify us immediately; you should not copy or use it for any purpose, nor disclose its contents to any other person. Thank you.

Re: [EXTERNAL] PREEMPT - A few important items

Luke Hamel <hamel@ecohealthalliance.org>

Mon 3/26/2018 8:28 AM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Tonie Rocke (b) (6) @gmail.com>; Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>; Jonathon Musser <musser@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>

Hi Tonie,

I hope you had a great trip. If you are able to begin drafting a budget justification, that would be very helpful. Whatever you cannot complete, we will be sure to get done.

Regarding the budget justification, I have reattached a template with appropriate headings and language that is already correctly formatted. **I would just ask you to insert the appropriate name/cost amount, substituting CAPITALIZED words and filling in gaps (indicated by underscores).**

Each section in the budget (e.g. Personnel, fringe, travel, etc.) should have a corresponding section in the budget justification (as shown in the template). Essentially, any line item that is listed in the budget needs to be justified in the 'budget justification' document.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
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On Mon, Mar 26, 2018 at 8:00 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Luke: I have returned from Mexico and just wading through email. Do you still need me to prepare a budget justification in a word document (everything was in the excel file) this AM? I'll get on it right away if it hasn't already been done. Please advise. Best -Tonie
>
> On Sat, Mar 24, 2018 at 1:29 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,

It looks as though we have a detailed budget from you, but we still will need a budget justification (essentially a Word doc. that provides justification for each line item of the budget).

Rachel and Katie - If you have time this weekend to get a start on the budget justification doc, that would be very helpful. If you're not available, which I understand may very well be the case, we will be happy to take this on. Please let us know.

Thank you,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Fri, Mar 23, 2018 at 4:46 PM, Tonie Rocke <(b) (6) @gmail.com> wrote:

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      - Long story short...there is NO need for you to identify any additional pricing assumptions.

Thank you and please let me know if you have any questions. I will be available by email and phone (mobile number listed below) over the weekend, should you need to contact me.

Best,

Luke Hamel
Program Assistant

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Rachel Abbott
USGS National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711
(608) 270-2489
Fax: (608) 270-2415

<PREEMPT_Eco_Impacts_Risk_Plan RCA.docx>

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

Budget Justification Template (Please follow the guidance in this template for each budget period)

PHASE 1

BASE PERIOD 1

A. Personnel ($Total)

- **NAME**, **PhD**, TITLE, will oversee all aspects….has in-depth knowledge and experience in…with expertise in…. We request $AMOUNT p.a. salary for NAME, who will dedicate # months p.a. to this project for phase ___ years ____.
- **NAME, PhD, TITLE**, will guide and advise…has worked with emerging zoonoses for over…. has experience managing…. We request $AMOUNT p.a. salary for NAME who will dedicate # months p.a. on this project for phase ____ years ____.
- **Post-doctoral fellow (TBD),** will help lead and coordinate all field and laboratory activities as well as data analyses and will dedicate # months p.a. to this project. We request $AMOUNT p.a. to cover stipend for a 3 year fellowship award.

B. Fringe ($Total)

Fringe benefits are calculated as per INSTITITION federally negotiated rate of ___% of base salary per year.

C. Travel ($Total)

Domestic Travel ($Total): We are requesting $AMOUNT in phase___ year___ to support domestic travel from ____to ____ for one (1) Co-PI/Co-I **and** one (1) research scientist to attend the **_____ meeting**. We calculate the expenses per person as follows: 2 economy, round-trip tickets (departure location <> return location) at $AMOUNT/person, # nights in hotel at $AMOUNT/person, and a total per diem allowance of $AMOUNT/person.

We are requesting $AMOUNT in the phase____year____ to support domestic travel from ____, to ____, for one (1) Co-PI/Co-I **and** one (1) research scientist to attend ____ meeting. We calculate the expenses per person as follows: 2 economy, round-trip tickets (departure <> return) at $AMOUNT/person, # nights in hotel at $AMOUNT/person, and a total per diem allowance of $AMOUNT/person.

International Travel ($Total): We request $AMOUNT p.a. for phase____ years____ to support international travel from Departure location to project study regions in LOCATION. We have budgeted for either a) one (1) Co-PI **and** two (2) research scientist; **or** b) three (3) research scientists to travel three

times to each region for ____ with local partners. Expenses per person for each trip are calculated as follows: 2 economy round trip tickets (ldeparture location<> return location) at $AMOUNT each, lodging at $AMOUNT x # nights, a total per diem allowance of $AMOUNT. Total estimated travel expenses to location per person per trip are $AMOUNT.

D. Field work ($Total)

Field team ($Total): We requesting $AMOUNT per year for phase____ years___ and $AMOUNT for phase___ year____ to cover stipends for # field assistants to conduct biological sampling.

Field visits ($Total): We are requesting $AMOUNT p.a. for phase___ years____and $AMOUNT for phase____ year____ to cover transportation to field sites.

E. Supplies and Materials ($Total)

We are requesting a total of **$AMOUNT** for supplies and materials across all phases and years. Expenses are calculated as follows:

- **Biological sampling supplies ($Total)** We are requesting $AMOUNT for phase___ year____ and $AMOUNT for phase____ year____ to purchase necessary supplies for biological sampling including (type, examples of) materials necessary for the collection (e.g. vials, swabs) and transportation of biological samples, and microscopes.
- **Computing devices ($Total)** 2 laptop computers at $AMOUNT for research analyses.
- **Office supplies ($Total):** We are requesting $AMOUNT to purchase office supplies to record biodiversity and laboratory data (notebooks, clipboards, pens, etc.).
- **Database development ($Total):** We request $AMOUNT for the development of an extensive, comprehensive database to store all collected data.
- **Publications ($Total):** We are requesting $AMOUNT p.a. for phase____ years____ to support journal publication costs of research results. We expect to produce ____ publications per year.
- **Internet ($Total):** Internet service for ___ months per year at $40.00 per month.
- **Cellphone service ($Total):** Cellphone service for ___ months per year at $AMOUNT per month.
- **Google apps for work ($Total):** Google apps service for __ months per year at $AMOUNT per month.
- **Printing and Photocopying ($1,620):** Printing and photocopying of survey instrument, survey guide and training materials.

F. Equipment ($Total)

We request a total of $AMOUNT in phase____ year____ to purchase ____ at $AMOUNT and ____ at $AMOUNT to preserve samples prior to shipment.

H. Indirect Costs ($Total)

We are requesting a federally negotiated indirect cost rate of ____% on all direct costs.

PHASE 1

BASE PERIOD 2

PHASE 2

OPTION PERIOD 1

PHASE 2

OPTION PERIOD 2

Re: [EXTERNAL] PREEMPT - A few important items

Luke Hamel <hamel@ecohealthalliance.org>

Mon 3/26/2018 9:48 AM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Tonie Rocke <(b) (6) @gmail.com>; Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>; Jonathon Musser <musser@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>

Thank you, Tonie! **Let's setup a tentative call for 4 PM (ET)/3 PM (CT).** We may not have any need for the call, but best to have one scheduled just in case.

Please use the following number and password to join the call:

Phone: 1-719-785-9461
Password: 9784#

Best,

Luke Hamel
Program Assistant

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On Mon, Mar 26, 2018 at 10:21 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Will do. I'll send a paragraph on facilities as well. Talk shortly. -Tonie
>
> On Mon, Mar 26, 2018 at 8:28 AM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > I hope you had a great trip. If you are able to begin drafting a budget justification, that would be very helpful. Whatever you cannot complete, we will be sure to get done.
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Each section in the budget (e.g. Personnel, fringe, travel, etc.) should have a corresponding section in the budget justification (as shown in the template). Essentially, any line item that is listed in the budget needs to be justified in the 'budget justification' document.

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<PREEMPT_Eco_Impacts_Risk_Plan RCA.docx>

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From: Rocke, Tonie <trocke@usgs.gov>
Sent: Monday, March 26, 2018 11:58 AM
To: Luke Hamel
Cc: Tonie Rocke; Abbott, Rachel; Richgels, Katherine; Jonathon Musser; Evelyn Luciano
Subject: Re: [EXTERNAL] PREEMPT - A few important items
Attachments: Budget Justification_rocke.docx; NWHC_BudgetBreakdown_26March2018-ter.xlsx; USGS_DEFUSE_budget_26Mar_ter.pdf

Hello Luke: Attached is my budget justification (word and updated excel files) and my final budget. I found several mistakes in the original budget (miscalculations in the travel budget) which I fixed and also when time was added to my salary, the fringe was not adjusted. Thus the budget is slightly different but not by much. I think I have caught everything and it all adds up now, but feel free to check. I will send facility description along soon. Thanks -Tonie

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Budget Justification Template (Please follow the guidance in this template for each budget period)

PHASE 1

BASE PERIOD 1

A. Personnel ($94,967)

- **Tonie E. Rocke**, **PhD**, Research Microbiologist, will oversee all aspects of developing methods to deliver immunomodulatory and immunoboosting agents to bats. Dr. Rocke has in-depth knowledge and experience in developing oral vaccines for use in controlling disease in wild animals, with particular expertise in developing vaccines for bats. We request $9,179 in salary for Dr. Tonie Rocke, who will dedicate 0.85 months p.a. to this project for phase1, year 1. An additional 1 month of Dr. Rocke's time will be provided in-kind.
- **Dr. Rachel Abbot, DVM, MS, Research Associate**, will help coordinate all field and laboratory activities as well as data compilation and analyses. Dr. Abbott has considerable experience in managing several previous vaccine projects in wild rodents and bats. We request $61,006 in salary for Dr. Abbott who will dedicate 12 months p.a. on this project for phase 1 year 1.

- **Undergraduate student assistants:** Three students will be hired for 3 to assist with bat feeding and husbandry for a total of $24,782.

B. Fringe ($18,445)

Fringe benefits are calculated as per US Geological Survey federally negotiated rate of 30.84% of base salary per year for Dr. Rocke and 26.35% for Dr. Abbott. Students receive an hourly wage but no fringe benefits

C. Travel ($9707.25)

Domestic MeetingTravel ($4,007,25): We are requesting $1,011.25 in phase 1, year 1 to support domestic travel from Madison, WI to Arlington, VA for one (1) Co-PI/Co-I to attend the DARPA Kickoff Meeting. We calculate the expenses per person as follows: 1 economy, round-trip tickets (Madison, WI <> Arlington Virginia) at $333, 2 nights in hotel at 437.50, plus $120 for parking and taxi and a total per diem allowance of $120.75.

We are requesting $2,996 in the phase 1, year 1 to support domestic travel from Madison, WI, to New York, New York for one (1) Co-PI/Co-I and one (1) research scientist to attend the annual meeting. We calculate the expenses per person as follows: 2 economy, round-trip tickets (Madison, WI <> New York,

New York) at $333/person, 3 nights in hotel at $291/person, and a total per diem allowance of $222/person, plus $140 for parking and taxi fare.

Domestic Field visit ($2316): We are requesting $2,316 in phase 1 year 1 to support domestic travel form Madison, Wisconsin to a cave site in Upper Peninsula, Michigan for 3 people (1 co-PI, 2 technicians) to test delivery methods in bats. We calculate the expenses per person as follows: 4 nights in a hotel at $93/person, a total per diem allowance of $204/person, and gas and use of government vehicle at a cost $588.

International Field visit ($3384)

We have budgeted for one Co-PI to travel for a cave site visit with local partners in China in phase 1, year 1. Expenses are calculated as follows: 1 economy round trip ticket (Madison, WI<> Kunming, Yunnan, China) for $1370, $1029 for lodging at $147 x 7 nights, a total per diem allowance of $805, plus $180 for parking and taxi fare for each visit.

E. Supplies and Materials (21,982.52)

Expenses are calculated as follows:

Animal Handling supplies ($11,288.67 Total): We are requesting $11,288.67 for phase 1, Year 1 for bat handling and supplies for both field and laboratory studies to include: a Harp trap for collecting bats, bat caging materials, mealworms for feeding bats, bat wing bands, anti-parasite medications, and PPE for handling bats (Cut resistant gloves, Tyvek suits, Tyvek aprons, N95 respirators, and PAPR replacement covers).

- **Vaccine production and biological sampling supplies ($10,693.85 Total)** We are requesting $10,693.85 for phase 1, year 1 to purchase necessary supplies for vaccine production and biological sampling including cell culture flasks, Nunc cell factories, fetal bovine serum, DMEM medium, glycerin jelly, rhodamine B, hair collection bags, 96 well plates, pipette tips and other consumables

F. Equipment (none requested)

G. Other direct costs

Animal Care ($12,600 Total) We are requesting $12,600 for phase 1, year 1. This amount covers animal care and room costs for up to 60 bats for 120 days at $105/day in a BSL3 animal facility, including daily husbandry, gut-loading meal worms, cleaning cages, veterinary services and daily surcharge for room use.

Rabies prophylaxis shots ($4020) We are requesting $4020 for phase 1, year 1 to provide rabies prophylactic vaccination for 4 animal care staff/year. Rabies vaccination is required of all staff handling bats due to the high prevalence of rabies in bats.

H. Indirect Costs ($Total)

We are requesting a federally negotiated indirect cost rate of 64.54% on all direct costs.

PHASE 1

BASE PERIOD 2

A. Personnel ($92,267)

- **Tonie E. Rocke**, **PhD**, Research Microbiologist, will oversee all aspects of developing methods to deliver immunomodulatory and immunoboosting agents to bats. Dr. Rocke has in-depth knowledge and experience in developing oral vaccines for use in controlling disease in wild animals, with particular expertise in developing vaccines for bats. We request $6,479 salary for Dr. Tonie Rocke, who will dedicate 0.6 months to this project for phase1, year 2. An additional 1 month of Dr. Rocke's time will be provided in-kind.
- **Dr. Rachel Abbot, DVM, MS, Research Associate**, will help coordinate all field and laboratory activities as well as data compilation and analyses. Dr. Abbott has considerable experience in managing several previous vaccine projects in wild rodents and bats. We request $61,006 in salary for Dr. Abbott who will dedicate 12 months on this project for phase 1 year 2.
- **Undergraduate student assistants:** Three students will be hired for 3 to assist with bat feeding and husbandry for a total of $24,782.

B. Fringe ($17,968)

Fringe benefits are calculated as per US Geological Survey federally negotiated rate of 30.84% of base salary per year for Dr. Rocke and 26.35% for Dr. Abbott. Students receive an hourly wage but no fringe benefits.

C. Travel ($10,561)

Domestic Field visit ($2316): We are requesting $2,316 in phase 1 year 1 to support domestic travel form Madison, Wisconsin to a cave site in Upper Peninsula, Michigan for 3 people (1 co-PI, 2 technicians) to test delivery methods in bats. We calculate the expenses per person as follows: 4 nights in a hotel at $93/person, a total per diem allowance of $204/person, and gas and use of government vehicle at a cost $588.

International Travel ($8245.50): We request $8,245.50 p.a. for phase 1, year 2 to support international travel from Madison, WI location to Annual Meeting in Wuhan, China. We calculate the expenses as follows: 1 economy, round trip ticket (Madison, WI<>Wuhan, China at $6,861, 5 nights hotel at $139.65, and a total per diem allowance of 546.25, plus $140 for parking and taxi fare.

E. Supplies and Materials (17,976.52)

Expenses are calculated as follows:

Animal Handling supplies ($7,282.67 Total): We are requesting $7,282.67 for phase 1, year 2 for bat handling and supplies for both field and laboratory studies to include bat caging materials, mealworms for feeding bats, bat wing bands, anti-parasite medications, and PPE for handling bats (Cut resistant gloves, Tyvek suits, Tyvek aprons, N95 respirators, and PAPR replacement covers).

- **Vaccine production and biological sampling supplies ($10,693.85 Total)** We are requesting $19,311.85 for phase 1, year 1-2, and phase 2, year 3 to purchase necessary supplies for vaccine production and biological sampling including cell culture flasks, Nunc cell factories, fetal bovine serum, DMEM medium, glycerin jelly, rhodamine B, hair collection bags, 96 well plates, pipette tips and other consumables

F. Equipment (none requested)

G. Other direct costs

Animal Care ($12,600 Total) We are requesting $12,600 for phase 1, year 1. This amount covers animal care and room costs for up to 60 bats for 120 days at $105/day in a BSL3 animal facility, including daily husbandry, gut-loading meal worms, cleaning cages, veterinary services and daily surcharge for room use.

Rabies prophylaxis shots ($4020) We are requesting $4020 for phase 1, year 1 to provide rabies prophylactic vaccination for 4 animal care staff/year. Rabies vaccination is required of all staff handling bats due to the high prevalence of rabies in bats.

H. Indirect Costs ($Total)

We are requesting a federally negotiated indirect cost rate of 64.54% on all direct costs.

PHASE 2

OPTION PERIOD 1

A. Personnel ($94,427)

- **Tonie E. Rocke**, **PhD**, Research Microbiologist, will oversee all aspects of developing methods to deliver immunomodulatory and immunoboosting agents to bats. Dr. Rocke has in-depth knowledge and experience in developing oral vaccines for use in controlling disease in wild animals, with particular expertise in developing vaccines for bats. We request $8,639 in salary for

Dr. Tonie Rocke, who will dedicate 0.8 months p.a. to this project for phase 2, option year 1. An additional 1 month of Dr. Rocke's time will be provided in-kind.

- **Dr. Rachel Abbot, DVM, MS, Research Associate**, will help coordinate all field and laboratory activities as well as data compilation and analyses. Dr. Abbott has considerable experience in managing several previous vaccine projects in wild rodents and bats. We request $61,006 in salary for Dr. Abbott who will dedicate 12 months p.a. on this project for phase 2, Option year 1

- **Undergraduate student assistants:** Three students will be hired for 3 to assist with bat feeding and husbandry for a total of $24,782.

B. Fringe ($18,634)

Fringe benefits are calculated as per US Geological Survey federally negotiated rate of 30.84% of base salary per year for Dr. Rocke and 26.35% for Dr. Abbott. Students receive an hourly wage but no fringe benefits

C. Travel ($11,430)

Domestic MeetingTravel ($2996):

We are requesting $2,996 in the phase 2, option year 1 to support domestic travel from Madison, WI, to New York, New York for one (1) Co-PI/Co-I and one (1) research scientist to attend the annual meeting. We calculate the expenses per person as follows: 2 economy, round-trip tickets (Madison, WI <> New York, New York) at $333/person, 3 nights in hotel at $291/person, and a total per diem allowance of $222/person, plus $140 for parking and taxi fare.

Domestic Field visit ($2316): We are requesting $2,316 in phase 2, option year 1 to support domestic travel from Madison, Wisconsin to a cave site in Upper Peninsula, Michigan for 3 people (1 co-PI, 2 technicians) to test delivery methods in bats. We calculate the expenses per person as follows: 4 nights in a hotel at $93/person, a total per diem allowance of $204/person, and gas and use of government vehicle at a cost $588.

International Field visit ($6118)

We have budgeted for one Co-PI and one research associate to travel for a cave site visit with local partners in China in phase 2, option year1. Expenses per person are calculated as follows: 1 economy round trip ticket (Madison, WI<> Kunming, Yunnan, China) for $1370, $1029 for lodging at $147 x 7 nights, a total per diem allowance of $805. An additional $180 is requested for parking and taxi fare.

E. Supplies and Materials ($17,976.52)

Expenses are calculated as follows:

Animal Handling supplies ($7,282.67 Total): We are requesting $7,282.67 for phase 2, option year 1 for bat handling and supplies for both field and laboratory studies to include: bat caging materials, mealworms for feeding bats, bat wing bands, anti-parasite medications, and PPE for handling bats (Cut resistant gloves, Tyvek suits, Tyvek aprons, N95 respirators, and PAPR replacement covers).

- **Vaccine production and biological sampling supplies ($10,693.85 Total)** We are requesting $10,693.85 for phase 2, option year 1 to purchase necessary supplies for vaccine production and biological sampling including cell culture flasks, Nunc cell factories, fetal bovine serum, DMEM medium, glycerin jelly, rhodamine B, hair collection bags, 96 well plates, pipette tips and other consumables

F. Equipment (none requested)

G. Other direct costs

Animal Care ($12,600 Total) We are requesting $12,600 for phase 2, option year 1. This amount covers animal care and room costs for up to 60 bats for 120 days at $105/day in a BSL3 animal facility, including daily husbandry, gut-loading meal worms, cleaning cages, veterinary services and daily surcharge for room use.

Rabies prophylaxis shots ($4020) We are requesting $4020 for phase 2, option year 1 to provide rabies prophylactic vaccination for 4 animal care staff/year. Rabies vaccination is required of all staff handling bats due to the high prevalence of rabies in bats.

H. Indirect Costs ($Total)

We are requesting a federally negotiated indirect cost rate of 64.54% on all direct costs.

PHASE 2

OPTION PERIOD 2

A. Personnel ($34,821)

- **Tonie E. Rocke, PhD**, Research Microbiologist, will oversee all aspects of developing methods to deliver immunomodulatory and immunoboosting agents to bats. Dr. Rocke has in-depth knowledge and experience in developing oral vaccines for use in controlling disease in wild animals, with particular expertise in developing vaccines for bats. We request $9,179 in salary for Dr. Tonie Rocke, who will dedicate 0.4 months to this project for phase 2, option period 2.
- **Dr. Rachel Abbot, DVM, MS, Research Associate**, will help coordinate all field and laboratory activities as well as data compilation and analyses. Dr. Abbott has considerable experience in

managing several previous vaccine projects in wild rodents and bats. We request $61,006 in salary for Dr. Abbott who will dedicate 6 months. on this project for phase 2, option period 2.

B. Fringe ($9,318)

Fringe benefits are calculated as per US Geological Survey federally negotiated rate of 30.84% of base salary per year for Dr. Rocke and 26.35% for Dr. Abbott.

C. Travel ($3501)

Domestic MeetingTravel ($3501):

We are requesting $3,501 in the phase 2, optional period 2 to support domestic travel from Madison, WI, to New York, New York for one (1) Co-PI/Co-I and one (1) research scientist to attend the annual meeting. We calculate the expenses per person as follows: 2 economy, round-trip tickets (Madison, WI <> New York, New York) at $333/person, 4 nights in hotel at $291 and a total per diem allowance of $296, plus $140 for parking and taxi fare for Co-PI and 3 nights in a hotel at $291 for research associate, total per diem allowance of $222 and $140 for parking and taxi fare.

E. Supplies and Materials ($2,163.43)

Expenses are calculated as follows:

Biological sampling supplies ($2,163,43 Total) We are requesting $2,163,43 for phase 2, option period 2 to purchase supplies to finish up biological sampling, including 96 well plates, pipette tips and other consumables.

F. Equipment (none requested)

TRAVEL

Trip #:	1	Location:	Arlington, VA, USA			Contract Period
Purpose:	DARPA Kickoff Meeting					Base 1

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
1.75	1	$333.00	$69.00	$250.00	$120.00	$1,011.25

Itemized Expenses for Other

Description	Amount
Parking	$20.00
Transportation to/from airport and in Arlington	$100.00
Total:	$120.00

Trip #:	2	Location:	Kunming, Yunnan, China			Contract Period
Purpose:	China Cave Site Visit					Base 1

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
7	1	$1,370.00	$115.00	$147.00	$180.00	$3,384.00

Itemized Expenses for Other

Description	Amount
Parking	$80.00
Transportation to/from airport and in Arlington	$100.00
Total:	$180.00

Trip #:	3	Location:	Upper Peninsula Michagan			Contract Period
Purpose:	US Cave Site Visit					Base 1

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
4	3	$0.00	$51.00	$93.00	$588.00	$2,316.00

Itemized Expenses for Other

Description	Amount
Gas	$120.00
Government Car Use	$468.00
Total:	$588.00

Trip #:	4	Location:	New York, NY, USA			Contract Period
Purpose:	Annual Meeting (Rocke + Abbott)					Base 1

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
3	2	$333.00	$74.00	$291.00	$140.00	$2,996.00

Itemized Expenses for Other

Description	Amount
Parking	$40.00
Transportation to/from airport and in New York	$100.00
Total:	$140.00

Trip #:	5	Location:	Upper Peninsula, Michigan, USA			Contract Period
Purpose:	US Cave Site Visit					Base 2

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
4	3	$0.00	$51.00	$93.00	$588.00	$2,316.00

Itemized Expenses for Other

Description	Amount
Gas	$120.00
Government Car Use	$468.00
Total:	$588.00

Trip #:	6	Location:	Wuhan, China			Contract Period
Purpose:	Annual Meeting (Rocke)					Base 2

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
4.75	1	$6,861.00	$115.00	$147.00	$140.00	$8,245.50

Itemized Expenses for Other

Description	Amount
Parking	$40.00
Transportation to/from airport in Wuhan	$100.00
Total:	$140.00

Trip #:	7	Location:	Upper Peninsula, Michigan, USA			Contract Period
Purpose:	US Cave Site Visit					Option I

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
4	3	$0.00	$51.00	$93.00	$588.00	$2,316.00

Itemized Expenses for Other

Description	Amount
Gas	$120.00
Government Car Use	$468.00
Total:	$588.00

Trip #:	8	Location:	Kunming, Yunnan, China			Contract Period
Purpose:	Deployment Visit					Option I

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
7	2	$1 370.00	$115.00	$147.00	$180.00	$6 588.00

Itemized Expenses for Other

Description	Amount
Parking	$80.00
Transportation to/from airport and in Kunming	$100.00
Total:	$180.00

Trip #:	9	Location:	New York, NY, USA			Contract Period
Purpose:	Annual Meeting (Rocke + Abbott)					Option I

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
3	2	$666.00	$74.00	$291.00	$140.00	$2,996.00

Itemized Expenses for Other

Description	Amount
Parking	$40.00
Transportation to/from airport	$100.00
Total:	$140.00

Trip #:	10	Location:	New York, NY, USA			Contract Period
Purpose:	Annual Meeting (Rocke + Abbott)					Option II

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem	Other	Total
4	1	$333.00	$74.00	$291.00	$140.00	$1,933.00
3	1	$333.00	$74.00	$291.00	$140.00	$1,568.00

Itemized Expenses for Other

Description	Amount
Parking	$40.00
Transportation to/from airport and in New York	$100.00
Total:	$140.00

MATERIALS/EQUIPMENT

Item	Manufacturer	Part Number	Unit Price	Quantity	Total Price	Contract Period	Additional Information
Harp Trap	Bat conservation and management		$2,003	2	$4,006 00	Y1	
Mealworms	Rainbow mealworms		$100/20,000	12	$1,200 00	Y1-Y3	
bat caging materials	various		$500/cage	9	$4,500 00	Y1-Y3	custom made
bat wing bands	Porzana		$596/box	9	$4,768 00	Y1-Y3	
Cut resistant gloves	Varied		$15/pr	30	$450 00	Y1-Y3	
Tyvek suits	DuPOnt	EV29135313	$306/case	15	$4,590 00	Y1-Y3	
Tyvek aprons	Lakeland	6EHH7	$58/case	15	$870 00	Y1-Y3	
N95 respirators	3M	9511	$20/box	45	$900 00	Y1-Y3	
PAPRs replacement covers	3M		$96/3 units	45	$4,320 00	Y1-Y3	
Selamectin	Zoetis		$250		$250 00	Y1-Y3	
cell culture flasks	Corning	430641U	415/case	5	$2,075 00	Y1-Y3	
cell culture flasks	Corning	431080	425/case	10	$4,250 00	Y1-Y3	
Nunc cell factories	Nunc	140250	$370/case	12	$4,440 00	Y1-Y3	
fetal bovine serum	GE Hyclone	SH30071 03	$600/bottle	8	$4,800 00	Y1-Y3	
DMEM medium	GE Hyclone	SH30021 02	$30/l	10	$300 00	Y1-Y3	
glycerin jelly	Carolina Biological Supply		$43 bottle	50	$2,150 00	Y1-Y3	
rhodamine B	Sigma		$56/100g	6	$336 00	Y1-Y3	
hair collection bags	U-line		$75/box	10	$750 00	Y1-Y3	
96 well plates	Corning	3599	$600/case	8	$4,800 00	Y1-Y3 5	
pipette tips	Fisher	13-676-10	$100/case	50	$5,000 00	Y1-Y3 5	
Consumables	miscellaneous				$5,344 00	Y1-Y3 5	needles, syringes,whirl paks, plastic bags, other disposables, all <5K
				Total	$60,099 00		
				Y1 Total	$25,854.00	$21,982 52	
				Y2 Total		$17,976 52	
				Y3 Total		$17,976 52	
				Y3 5 Total		$2,163 43	
						$60,098.99	

MATERIALS/EQUIPMENT

Item	Manufacturer	Part Number	Unit Price	Quantity	Total Price	Contract Period	Additional Information
Harp Trap	Bat conservation and management		$2,003	2	$4,006 00	Y1	
Mealworms	Rainbow mealworms		$100/20,000	12	$1,200 00	Y1-Y3	
bat caging materials	various		$500/cage	9	$4,500 00	Y1-Y3	custom made
bat wing bands	Porzana		$596/box	9	$4,768 00	Y1-Y3	
Cut resistant gloves	Varied		$15/pr	30	$450 00	Y1-Y3	
Tyvek suits	DuPOnt	EV29135313	$306/case	15	$4,590 00	Y1-Y3	
Tyvek aprons	Lakeland	6EHH7	$58/case	15	$870 00	Y1-Y3	
N95 respirators	3M	9511	$20/box	45	$900 00	Y1-Y3	
PAPRs replacement covers	3M		$96/3 units	45	$4,320 00	Y1-Y3	
Selamectin	Zoetis		$250		$250 00	Y1-Y3	
cell culture flasks	Corning	430641U	415/case	5	$2,075 00	Y1-Y3	
cell culture flasks	Corning	431080	425/case	10	$4,250 00	Y1-Y3	
Nunc cell factories	Nunc	140250	$370/case	12	$4,440 00	Y1-Y3	
fetal bovine serum	GE Hyclone	SH30071 03	$600/bottle	8	$4,800 00	Y1-Y3	
DMEM medium	GE Hyclone	SH30021 02	$30/l	10	$300 00	Y1-Y3	
glycerin jelly	Carolina Biological Supply		$43 bottle	50	$2,150 00	Y1-Y3	
rhodamine B	Sigma		$56/100g	6	$336 00	Y1-Y3	
hair collection bags	U-line		$75/box	10	$750 00	Y1-Y3	
96 well plates	Corning	3599	$600/case	8	$4,800 00	Y1-Y3 5	
pipette tips	Fisher	13-676-10	$100/case	50	$5,000 00	Y1-Y3 5	
Consumables	miscellaneous				$5,344 00	Y1-Y3 5	needles, syringes,whirl paks, plastic bags, other disposables, all <5K
				Total	$60,099 00		
				Y1 Total	$25,854.00	$21,982 52	
				Y2 Total		$17,976 52	
				Y3 Total		$17,976 52	
				Y3 5 Total		$2,163 43	
						$60,098.99	

WORKSPACE FORM

1-800-518-4726
SUPPORT@GRANTS.GOV

This Workspace form is one of the forms you need to complete prior to submitting your Application Package. This form can be completed in its entirety offline using Adobe Reader. You can save your form by clicking the "Save" button and see any errors by clicking the “Check For Errors” button. In-progress and completed forms can be uploaded at any time to Grants.gov using the Workspace feature.

When you open a form, required fields are highlighted in yellow with a red border. Optional fields and completed fields are displayed in white. If you enter invalid or incomplete information in a field, you will receive an error message. Additional instructions and FAQs about the Application Package can be found in the Grants.gov Applicants tab.

OPPORTUNITY & PACKAGE DETAILS:

Opportunity Number:	HR001118S0017
Opportunity Title:	PREventing EMerging Pathogenic Threats
Opportunity Package ID:	PKG00237724
CFDA Number:	12.910
CFDA Description:	Research and Technology Development
Competition ID:	
Competition Title:	
Opening Date:	01/19/2018
Closing Date:	03/27/2018
Agency:	DARPA - Biological Technologies Office
Contact Information:	BAA Coordinator PREEMPT@darpa.mil

APPLICANT & WORKSPACE DETAILS:

Workspace ID:	WS00094394
Application Filing Name:	Project DEFUSE
DUNS:	0770900660000
Organization:	ECOHEALTH ALLIANCE INC.
Form Name:	R & R Subaward Budget 10 YR Subform
Form Version:	1.4
Subform Name:	USGS Ntl. Wildlife Health Cen
Requirement:	Optional
Download Date/Time:	Mar 06, 2018 05:28:38 PM EST
Form State:	Error(s)

FORM ACTIONS:

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period:** 1 **Start Date:** 12/01/2018 **End Date:** 11/30/2019

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.85			9,179.00	2,475.00	11,654.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 88,630.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00	0.00	24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel: 24,782.00

Total Salary, Wages and Fringe Benefits (A+B): 113,412.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: [ ] Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	6,323.25
2. Foreign Travel Costs	3,384.00
Total Travel Cost	9,707.25

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	21,982.52
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.	Rabies prophylaxis	4,020.00
10.		
	Total Other Direct Costs	38,602.52

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	161,721.77

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	161,721.77	104,375.23

Total Indirect Costs 104,375.23

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	266,097.00

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	266,097.00

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 2** **Start Date:** 12/01/2019 **End Date:** 11/30/2020

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.60			6,479.00	1,998.00	8,477.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: [Add Attachment] [Delete Attachment] [View Attachment]

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 85,453.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00	0.00	24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel: 24,782.00

Total Salary, Wages and Fringe Benefits (A+B): 110,235.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: [ ] Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	2,316.00
2. Foreign Travel Costs	8,245.50
Total Travel Cost	10,561.50

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	17,976.52
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.	Rabies prophylaxis	4,020.00
10.		
	Total Other Direct Costs	34,596.52

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	155,393.02

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	155,393.02	100,290.65

Total Indirect Costs 100,290.65

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	255,683.67

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	255,683.67

L. Budget Justification

(Only attach one file.) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 3** **Start Date:** 12/01/2020 **End Date:** 11/30/2021

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.80			8,639.00	2,664.00	11,303.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: [Add Attachment] [Delete Attachment] [View Attachment]

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 88,279.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00	0.00	24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel: 24,782.00

Total Salary, Wages and Fringe Benefits (A+B): 113,061.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: [ ] Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	5,312.00
2. Foreign Travel Costs	6,118.00
Total Travel Cost	11,430.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees — **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	17,976.52
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.	Rabies prophylaxis	4,020.00
10.		
	Total Other Direct Costs	34,596.52

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	159,087.52

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	158,087.52	102,029.68

Total Indirect Costs	102,029.68

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	261,117.20

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	261,117.20

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 4

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 4** **Start Date:** 12/01/2021 **End Date:** 03/31/2022

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.40			4,319.00	1,332.00	5,651.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	6.00			30,502.00	7,986.00	38,488.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: [Add Attachment] [Delete Attachment] [View Attachment]

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 44,139.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						

Total Number Other Personnel

Total Other Personnel

Total Salary, Wages and Fringe Benefits (A+B): 44,139.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	3,501.00
2. Foreign Travel Costs	
Total Travel Cost	3,501.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	2,163.43
2.	Publication Costs	6,000.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.		
9.		
10.		
	Total Other Direct Costs	8,163.43

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	55,803.43

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	55,803.43	36,015.26

Total Indirect Costs	36,015.26

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	91,818.69

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	91,818.69

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		306,501.00
Section B, Other Personnel		74,346.00
Total Number Other Personnel	9	
Total Salary, Wages and Fringe Benefits (A+B)		380,847.00
Section C, Equipment		
Section D, Travel		35,199.75
1. Domestic	17,452.25	
2. Foreign	17,747.50	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		115,958.99
1. Materials and Supplies	60,098.99	
2. Publication Costs	6,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	37,800.00	
9. Other 2	12,060.00	
10. Other 3		
Section G, Direct Costs (A thru F)		532,005.74
Section H, Indirect Costs		342,710.82
Section I, Total Direct and Indirect Costs (G + H)		874,716.56
Section J, Fee		
Section K, Total Costs and Fee (I + J)		874,716.56

Re: [EXTERNAL] PREEMPT - A few important items

Luke Hamel <hamel@ecohealthalliance.org>

Mon 3/26/2018 2:01 PM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Tonie Rocke <(b) (6) @gmail.com>; Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>; Jonathon Musser <musser@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>

Excellent. Thank you for all of your work on this, Tonie! I will speak with Jonathon and address your question regarding the DARPA kick-off meeting.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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(b) (6) (mobile)
www.ecohealthalliance.org

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> >
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substituting CAPITALIZED words and filling in gaps (indicated by underscores).

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> >   - This being said - Rachel and Katherine, could you please write-up a short section (a paragraph or so), that

addresses this issue 'long-term safety and efficacy'?
- I apologize for the extremely short notice, but **we would greatly appreciate it if you could return this to us by tomorrow afternoon, Sat. (3/24).**

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(608) 270-2489
Fax: (608) 270-2415

<PREEMPT_Eco_Impacts_Risk_Plan RCA.docx>

--
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Re: [EXTERNAL] PREEMPT - A few important items

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Mon 3/26/2018 2:16 PM

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<PREEMPT_Eco_Impacts_Risk_Plan RCA.docx>

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The National Wildlife Center (NWHC) is located on 24 acres of Federal property in Madison, Wisconsin. The facility was designed to meet all of the criteria set down by the National Institutes of Health (NIH) and the Center for Disease Control for Biological Safety Level III (BSL-III) research. The research building, of approximately 32,000 square feet, contains specialized research laboratories and support areas, staff offices, and BSL-III biocontainment animal research areas. Two, fully equipped laboratories in the research building are available at all times for the proposed work. Each laboratory is equipped with an autoclave and has two adjacent isolation rooms supplemented with biosafety cabinets for handling of specimens and cultures. The laboratories and containment rooms are maintained under negative air pressure at all times. Animal research involving infectious agents is performed within the NWHC's BSL-III biocontainment animal research area, or Animal Isolation Wing (AIW). Staff must go through a complete clothing change to enter the AIW and are required to pass through an automatically activated shower before leaving the containment area. The AIW is equipped with pathology incineration and steam sterilization equipment, and an ultraviolet radiation chamber so that all materials can be treated before leaving the biological containment area, and the area is maintained under negative air pressure. Animal care staff is available and trained to maintain, monitor and handle animals as required. A Veterinary Medical Officer is on site to address any animal health issues, and the AIW is fully equipped for medical and surgical procedures.

Re: [EXTERNAL] PREEMPT - A few important items

Luke Hamel <hamel@ecohealthalliance.org>

Mon 3/26/2018 2:32 PM

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Hi Tonie,

Thank you for the facility description. There won't be a need for us to have a call, but please review the draft and look for ways to reduce the text of your technical section.

Additionally, I was hoping to confirm the following two points:

(1) Is NWHC's CAGE code the following? **52Y40**

(2) Which of the following 'organization types', best describes NWHC?

-"LARGE BUSINESS", "SMALL DISADVANTAGED BUSINESS", "OTHER SMALL BUSINESS", "HBCU", "MI", "OTHER EDUCATIONAL", OR "OTHER NONPROFIT";

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
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On Mon, Mar 26, 2018 at 8:00 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

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> Best -Tonie
>
> On Sat, Mar 24, 2018 at 1:29 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
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> > Rachel and Katie - If you have time this weekend to get a start on the budget justification doc, that would be very helpful. If you're not available, which I understand may very well be the case, we will be happy to take this on. Please let us know.
> >
> > Thank you,
> >
> > **Luke Hamel**
> > *Program Assistant*
> >
> > EcoHealth Alliance
> > 460 West 34th Street – 17th floor
> > New York, NY 10001
> >
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On Fri, Mar 23, 2018 at 4:46 PM, Tonie Rocke (b) (6) @gmail.com> wrote:

> Hello all: I sent my budget justification to Ana several days ago. Thanks for addressing safety issues Rachel!
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> On Mar 23, 2018, at 2:38 PM, Abbott, Rachel <rabbott@usgs.gov> wrote:
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> > I have added some paragraphs to the page you sent. It just deals with safety of RCN, so I hope that is enough. Most of the text came out of documents we have to write to get approval to use our RCN vaccines in the field (risk analysis for USDA CVB and environmental assessment for USGS). Unfortunately, as I said before, I'll be unavailable until next Thursday, but Tonie should be back in her office on Monday.
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> >
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> > Hi Rachel and Katherine,
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> > I wanted to address a few important PREEMPT items with you:
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> > - Regarding **NWHC budget justification**
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> > - Regarding language on **'Long-term safety and efficacy'**
> > - In the PREEMPT proposal, we must state how we will establish methods to assess the 'long-term safety and efficacy of our preemptive approaches'
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> > - I have attached language from the BAA to provide you with further guidance on what DARPA requires us to include.
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> > - Regarding '**pricing assumptions**' for NWHC facilities

- Previously, we had asked you to identify any 'pricing assumptions' that may correspond with use of government facilities. Due to confusion about what exactly was being asked for, we reached out to DARPA staff, asking them to clarify the matter:
  - We asked: "EcoHealth Alliance has a USG entity listed as a subcontractor in our proposal. Is the USG entity required to identify any pricing assumptions beyond those within their fully detailed and documented budget?
  - To which they responded: "No"
    - Long story short...there is NO need for you to identify any additional pricing assumptions.

Thank you and please let me know if you have any questions. I will be available by email and phone (mobile number listed below) over the weekend, should you need to contact me.

Best,

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Program Assistant

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Rachel Abbott
USGS National Wildlife Health Center
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Madison, WI 53711
(608) 270-2489
Fax: (608) 270-2415

<PREEMPT_Eco_Impacts_Risk_Plan RCA.docx>

[EXTERNAL] PREEMPT - 'Intellectual Property'

Luke Hamel <hamel@ecohealthalliance.org>

Mon 3/26/2018 5:20 PM

To: Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; 周鹏 (peng.zhou@wh.iov.cn) <peng.zhou@wh.iov.cn>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Sims, Amy C <sims0018@email.unc.edu>; Sheahan, Timothy Patrick <sheahan@email.unc.edu>; wang linfa <linfa.wang@duke-nus.edu.sg>; Danielle Anderson <danielle.anderson@duke-nus.edu.sg>; Aaron Trent Irving <aaron.irving@duke-nus.edu.sg>; Rocke, Tonie E <trocke@usgs.gov>; Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>
Cc: Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Kateri.Paul@parc.com <Kateri.Paul@parc.com>; Richgels, Katherine L <krichgels@usgs.gov>; Baric, Toni C <antoinette_baric@med.unc.edu>; Evelyn Luciano <luciano@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>

Hi all,

The PREEMPT BAA states that "All proposers must provide a good faith representation that the proposer either owns or possesses the appropriate licensing rights to all intellectual property that will be utilized under the proposed effort."

Could you **please read through the attached document and fill in the table**, as it applies to your institution? Please return to me as soon as possible.

Thank you, and please let me know if you have any questions.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
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New York, NY 10001

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Intellectual Property

All proposers must provide a good faith representation that the proposer either owns or possesses the appropriate licensing rights to all intellectual property that will be utilized under the proposed effort.

For All Non-Procurement Contracts

Proposers responding to this BAA requesting a Grant, Cooperative Agreement, Technology Investment Agreement, or Other Transaction for Prototypes shall follow the applicable rules and regulations governing these various award instruments, but, in all cases, **should appropriately identify any potential restrictions on the Government's use of any Intellectual Property contemplated under the award instrument in question**. This includes both Noncommercial Items and Commercial Items. Proposers are encouraged to use a format similar to that described in the section below. **If no restrictions are intended, then the proposer should state "NONE."**

Technical data computer software to be furnished with restrictions	Summary of intended use in the conduct of research	Basis for assertion	Asserted rights category	Name of person asserting rights
(LIST)	(NARRATIVE)	(LIST)	(LIST)	(LIST)

[EXTERNAL] Re: CAGE Code

Luke Hamel <hamel@ecohealthalliance.org>

Mon 3/26/2018 8:33 PM

To: Rocke, Tonie E <trocke@usgs.gov>

Thank you, Tonie.

Best,

Luke Hamel
Program Assistant

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On Mon, Mar 26, 2018 at 5:09 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Here is what I learned about our CAGE Code; it's different than what you had. -T
> ---------- Forwarded message ----------
> From: **Hankins, Thomas** <thankins@usgs.gov>
> Date: Mon, Mar 26, 2018 at 4:07 PM
> Subject: CAGE Code
> To: Tonie Rocke <trocke@usgs.gov>
> Cc: Lisa Meicher <lmeicher@usgs.gov>
>
> Tonie,
>
> Per a 10/2017 listing I found, our CAGE Code is 3VXB0
> main USGS is 1AW56
>
> Tom Hankins
> Administrative Officer
> USGS National Wildlife Health Center
> 6006 Schroeder Rd
> Madison, WI 53711
> 608-270-2412

> thankins@usgs.gov

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

Re: [EXTERNAL] PREEMPT - A few important items

Luke Hamel <hamel@ecohealthalliance.org>

Tue 3/27/2018 6:38 AM

To: Rocke, Tonie E <trocke@usgs.gov>

Hi Tonie,

HBCU stands for 'Historically Black College.University' but I am not sure what 'MI' stands for. I don't believe it would apply to your institution, however.

Luke Hamel
Program Assistant

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On Mon, Mar 26, 2018 at 7:41 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Do you know what HBCU or MI stands for? I did ask someone and they thought none of these fit either. I'll ask again. -T
>
> On Mon, Mar 26, 2018 at 6:33 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > Thank you for the information regarding the CAGE code. Unfortunately, there's no option listed for 'federal government research facility', but someone from your finance department should know which of these 'organization types' you fall under. I realize this answer may have to wait until morning, but please let us know as soon as you find out.
> >
> > Thank you,
> >
> > **Luke Hamel**
> > *Program Assistant*
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On Mon, Mar 26, 2018 at 3:44 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> What is a CAGE code? None of those organization types describe NWHC. We are a federal government research facility. Isn't there an option for that? -T
>
> On Mon, Mar 26, 2018 at 2:32 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > Thank you for the facility description. There won't be a need for us to have a call, but please review the draft and look for ways to reduce the text of your technical section.
> >
> > Additionally, I was hoping to confirm the following two points:
> > (1) Is NWHC's CAGE code the following? **52Y40**
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Hello again Luke: Here is a brief description of our facility. Let me know if this is sufficient. Also, will we be having a call or not? I have not yet had a chance to review the technical proposal but will do so shortly.

On Mon, Mar 26, 2018 at 2:01 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Excellent. Thank you for all of your work on this, Tonie! I will speak with Jonathon and address your question regarding the DARPA kick-off meeting.
>
> Best,
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> **Luke Hamel**
> *Program Assistant*
>
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> > > Hi Tonie,
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Regarding the budget justification, I have reattached a template with appropriate headings and language that is already correctly formatted. **I would just ask you to insert the appropriate name/cost amount, substituting CAPITALIZED words and filling in gaps (indicated by underscores).**

Each section in the budget (e.g. Personnel, fringe, travel, etc.) should have a corresponding section in the budget justification (as shown in the template). Essentially, any line item that is listed in the budget needs to be justified in the 'budget justification' document.

Best,

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- In the PREEMPT proposal, we must state how we will establish methods to assess the 'long-term safety and efficacy of our preemptive approaches'
  - Given your field of work, do you have any existing language on how to address potential negative impacts of intervention approaches on non-target species?
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    - To which they responded: "No"
      - Long story short...there is NO need for you to identify any additional pricing assumptions.

Thank you and please let me know if you have any questions. I will be available by email and phone (mobile number listed below) over the weekend, should you need to contact me.

Best,

Luke Hamel
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Rachel Abbott
USGS National Wildlife Health Center
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Fax: (608) 270-2415

<PREEMPT_Eco_Impacts_Risk_Plan RCA.docx>

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Re: [EXTERNAL] PREEMPT - A few important items

Luke Hamel <hamel@ecohealthalliance.org>

Tue 3/27/2018 6:52 AM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Jonathon Musser <musser@ecohealthalliance.org>; Daszak Peter <daszak@ecohealthalliance.org>; Tonie Rocke <(b) (6) @gmail.com>; Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>; Evelyn Luciano <luciano@ecohealthalliance.org>

Received. Thank you, Tonie!

Best,

Luke Hamel
Program Assistant

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On Tue, Mar 27, 2018 at 7:42 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Jonathan: Our DUNS number is 038975934. -Tonie
>
> On Tue, Mar 27, 2018 at 5:44 AM, Jonathon Musser <musser@ecohealthalliance.org> wrote:
>
> > Tonie, would you please confirm your DUNS number as soon as possible today?
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> > Thanks!
> > Jonathon
> >
> > On Mon, Mar 26, 2018 at 4:54 PM, Rocke, Tonie <trocke@usgs.gov> wrote:
> >
> > > Hi Luke: Attached are my comments on the full draft; I added my comments to Jerome's draft. I corrected a few errors, deleted at least one sentence in my section, and also added my deliverables for Task 7. I'm not certain what you want for project metrics. A timeline or something? Just repeating the deliverables doesn't seem appropriate. Also, I have someone checking on the CAGE code. Let me know if you need anything else. Thanks! -Tonie

On Mon, Mar 26, 2018 at 2:32 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

Hi Tonie,

Thank you for the facility description. There won't be a need for us to have a call, but please review the draft and look for ways to reduce the text of your technical section.

Additionally, I was hoping to confirm the following two points:

(1) Is NWHC's CAGE code the following? **52Y40**

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>
> > Hi Luke,
> > I have added some paragraphs to the page you sent. It just deals with safety of RCN, so I hope that is enough. Most of the text came out of documents we have to write to get approval to use our RCN vaccines in the field (risk analysis for USDA CVB and environmental assessment for USGS). Unfortunately, as I said before, I'll be unavailable until next Thursday, but Tonie should be back in her office on Monday.
> > --Rachel
> >
> > On Fri, Mar 23, 2018 at 1:45 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
> >
> > Hi Rachel and Katherine,
> >
> > I wanted to address a few important PREEMPT items with you:
> >
> > - Regarding **NWHC budget justification**
> >   - If you have not already done so (and I apologize for not knowing the answer), could you please send us your budget justification document? We are hoping to have all collaborator budgets and budget justifications as soon as possible.
> > - Regarding language on **'Long-term safety and efficacy'**
> >   - In the PREEMPT proposal, we must state how we will establish methods to assess the 'long-term safety and efficacy of our preemptive approaches'
> >   - Given your field of work, do you have any existing language on how to address potential negative impacts of intervention approaches on non-target species?
> >   - I have attached language from the BAA to provide you with further guidance on what DARPA requires us to include.
> >   - This being said - Rachel and Katherine, could you please write-up a short section (a paragraph or so), that addresses this issue 'long-term safety and efficacy'?

    - I apologize for the extremely short notice, but **we would greatly appreciate it if you could return this to us by tomorrow afternoon, Sat. (3/24).**
- Regarding '**pricing assumptions**' for NWHC facilities
    - Previously, we had asked you to identify any 'pricing assumptions' that may correspond with use of government facilities. Due to confusion about what exactly was being asked for, we reached out to DARPA staff, asking them to clarify the matter:
        - We asked: "EcoHealth Alliance has a USG entity listed as a subcontractor in our proposal. Is the USG entity required to identify any pricing assumptions beyond those within their fully detailed and documented budget?
        - To which they responded: "No"
            - Long story short...there is NO need for you to identify any additional pricing assumptions.

Thank you and please let me know if you have any questions. I will be available by email and phone (mobile number listed below) over the weekend, should you need to contact me.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
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New York, NY 10001

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--
Rachel Abbott
USGS National Wildlife Health Center
6006 Schroeder Road

Madison, WI 53711
(608) 270-2489
Fax: (608) 270-2415

<PREEMPT_Eco_Impacts_Risk_Plan RCA.docx>

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Jonathon Musser

PREDICT Program Assistant
EcoHealth Alliance Operations Team

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--

Tonie E. Rocke
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trocke@usgs.gov

Re: [EXTERNAL] IMPORTANT: DEFUSE grant proposal: indirect cost rate agreements needed ASAP

Molly Turner <turner@ecohealthalliance.org>

Tue 3/27/2018 8:41 AM

To: Luke Hamel <hamel@ecohealthalliance.org>
Cc: Rocke, Tonie E <trocke@usgs.gov>

Yes, thanks!

I'm wondering if you have something addressing fringe rates?

On Tue, Mar 27, 2018 at 9:37 AM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Excellent! Thank you very much, Tonie.
>
> Best,
>
> **Luke Hamel**
> *Program Assistant*
>
> EcoHealth Alliance
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> New York, NY 10001
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> ***EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.***
>
> On Tue, Mar 27, 2018 at 9:36 AM, Rocke, Tonie <trocke@usgs.gov> wrote:
>
> > Can't remember who asked for this, so I am sending it to both of you (IDC memo). See attached.-Tonie
> > ---------- Forwarded message ----------
> > From: **Meicher, Lisa** <lmeicher@usgs.gov>
> > Date: Tue, Mar 27, 2018 at 8:30 AM
> > Subject: Re: [EXTERNAL] IMPORTANT: DEFUSE grant proposal: indirect cost rate agreements needed ASAP
> > To: "Rocke, Tonie" <trocke@usgs.gov>
> > Cc: Thomas Hankins <thankins@usgs.gov>
> >
> > Morning Tonie,
> >
> > I attached the memo. I address the memo to EcoHealth Alliance, please let me know if that is not correct.

Lisa K. Meicher
Budget Analyst
USGS National Wildlife Health Center
6006 Schroeder Rd
Madison, WI 53711
608-270-2410
fax 608-270-2415
lmeicher@usgs.gov

On Mon, Mar 26, 2018 at 5:43 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Do we have a document regarding our indirect costs that I can send for a grant proposal. See request below. Thanks -Tonie
> ---------- Forwarded message ----------
> From: **Molly Turner** <turner@ecohealthalliance.org>
> Date: Mon, Mar 26, 2018 at 5:07 PM
> Subject: [EXTERNAL] IMPORTANT: DEFUSE grant proposal: indirect cost rate agreements needed ASAP
> To: trocke@usgs.gov, Jerome.Unidad@parc.com, "Baric, Ralph S" <rbaric@email.unc.edu>, linfa.wang@duke-nus.edu.sg
> Cc: rabbott@usgs.gov, sheahan@email.unc.edu, sims0018@email.unc.edu, danielle.anderson@duke-nus.edu.sg, Kateri.Paul@parc.com, Peter Daszak <daszak@ecohealthalliance.org>, Evelyn Luciano <luciano@ecohealthalliance.org>, Jonathon Musser <musser@ecohealthalliance.org>
>
> Dear DEFUSE team,
>
> Can you please provide an indirect cost rate agreement (current Forward Pricing Rate Agreement or Forward Pricing Rate Proposal if available, or if not available, 2 years historical data to include pool and expense costs used to generate the rates; or, for academia: DHHS or ONR negotiated rate package or, if calculated by other than a rate, provide University documentation identifying G&A and fringe costs by position) for your respective institutions ASAP (not later 4 pm EST tomorrow)?
>
> Thanks in advance and best regards,
> Molly Turner
> --
> **Molly Turner**
> *Federal Grants Coordinator*
> *EcoHealth Alliance Operations*
>
> EcoHealth Alliance
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--
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Molly Turner
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Re: [EXTERNAL] IMPORTANT: DEFUSE grant proposal: indirect cost rate agreements needed ASAP

Luke Hamel <hamel@ecohealthalliance.org>

Tue 3/27/2018 9:04 AM

To: Rocke, Tonie E <trocke@usgs.gov>

Unfortunately, the BAA states:

"Type of organization, **selected from among the following categories**: “LARGE BUSINESS,” “SMALL DISADVANTAGED BUSINESS,” “OTHER SMALL BUSINESS,” “HBCU,” “MI,” “OTHER EDUCATIONAL,” OR “OTHER NONPROFIT”;"

So it appears as though we can only choose from those categories listed.

Best,

Luke Hamel
Program Assistant

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On Tue, Mar 27, 2018 at 9:40 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Still working on the "organization type". Can we put "none of the above"?
>
> On Tue, Mar 27, 2018 at 8:37 AM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Excellent! Thank you very much, Tonie.
> >
> > Best,
> >
> > **Luke Hamel**
> > *Program Assistant*
> >
> > EcoHealth Alliance
> > 460 West 34th Street – 17th floor

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On Tue, Mar 27, 2018 at 9:36 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Can't remember who asked for this, so I am sending it to both of you (IDC memo). See attached.-Tonie
> ---------- Forwarded message ----------
> From: **Meicher, Lisa** <lmeicher@usgs.gov>
> Date: Tue, Mar 27, 2018 at 8:30 AM
> Subject: Re: [EXTERNAL] IMPORTANT: DEFUSE grant proposal: indirect cost rate agreements needed ASAP
> To: "Rocke, Tonie" <trocke@usgs.gov>
> Cc: Thomas Hankins <thankins@usgs.gov>
>
> Morning Tonie,
>
> I attached the memo. I address the memo to EcoHealth Alliance, please let me know if that is not correct.
>
> Lisa K. Meicher
> Budget Analyst
> USGS National Wildlife Health Center
> 6006 Schroeder Rd
> Madison, WI 53711
> 608-270-2410
> fax 608-270-2415
> lmeicher@usgs.gov
>
> On Mon, Mar 26, 2018 at 5:43 PM, Rocke, Tonie <trocke@usgs.gov> wrote:
>
> > Do we have a document regarding our indirect costs that I can send for a grant proposal. See request below. Thanks -Tonie
> > ---------- Forwarded message ----------
> > From: **Molly Turner** <turner@ecohealthalliance.org>
> > Date: Mon, Mar 26, 2018 at 5:07 PM
> > Subject: [EXTERNAL] IMPORTANT: DEFUSE grant proposal: indirect cost rate agreements needed ASAP
> > To: trocke@usgs.gov, Jerome.Unidad@parc.com, "Baric, Ralph S" <rbaric@email.unc.edu>, linfa.wang@duke-nus.edu.sg
> > Cc: rabbott@usgs.gov, sheahan@email.unc.edu, sims0018@email.unc.edu, danielle.anderson@duke-nus.edu.sg, Kateri.Paul@parc.com, Peter Daszak

<daszak@ecohealthalliance.org>, Evelyn Luciano <luciano@ecohealthalliance.org>, Jonathon Musser <musser@ecohealthalliance.org>

Dear DEFUSE team,

Can you please provide an indirect cost rate agreement (current Forward Pricing Rate Agreement or Forward Pricing Rate Proposal if available, or if not available, 2 years historical data to include pool and expense costs used to generate the rates; or, for academia: DHHS or ONR negotiated rate package or, if calculated by other than a rate, provide University documentation identifying G&A and fringe costs by position) for your respective institutions ASAP (not later 4 pm EST tomorrow)?

Thanks in advance and best regards,
Molly Turner
--
Molly Turner
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--
Tonie E. Rocke
USGS National Wildlife Health Center

> > 6006 Schroeder Rd.
> > Madison, WI 53711
> > 608-270-2451
> > trocke@usgs.gov

--
Tonie E. Rocke
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Re: [EXTERNAL] DEFUSE proposal: fringe rate for National Wildlife Health Center?

Molly Turner <turner@ecohealthalliance.org>

Tue 3/27/2018 9:39 AM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Anna Willoughby <willoughby@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>

Hey Tonie,

I've been asking around here and I'm not sure that we ever received the budget justification you're referring to. I'm so sorry if you sent already, but would you mind sharing again with all of us copied here?

Alternatively, if you can share with me a fringe rate agreement that supports this I think we've already done some work on pulling together a narrative to accompany the budget you shared, we so can just finish that up if you don't have something already prepared.

Thanks again for your quick responses at the 11th hour!

Best,
Molly

On Mon, Mar 26, 2018 at 7:00 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Oh sorry, you are right. I didn't read that correctly. Here is our fringe rates as I described in the budget justification. Fringe benefits are calculated as per US Geological Survey federally negotiated rate of 30.84% of base salary per year for Dr. Rocke and 26.35% for Dr. Abbott. Students receive an hourly wage but no fringe benefits
>
> On Mon, Mar 26, 2018 at 5:44 PM, Molly Turner <turner@ecohealthalliance.org> wrote:
>
> > Thanks so much Tonie, I think that is your IDC rate, what is the fringe rate? I see 26.4 listed but the actual fringe amounts your team has provided seem to vary.
> >
> > On Mon, Mar 26, 2018 at 6:40 PM, Rocke, Tonie <trocke@usgs.gov> wrote:
> >
> > > 64.54%
> > >
> > > On Mon, Mar 26, 2018 at 5:14 PM, Molly Turner <turner@ecohealthalliance.org> wrote:
> > >
> > > > Hi Tonie, Rachel, and Katie,
> > > >
> > > > I'm hoping one of you might be able to give me a quick answer as to the fringe rate your institution is using for your proposed DEFUSE budget?
> > > >
> > > > Thanks in advance and best regards,
> > > > Molly Turner
> > > >
> > > > --
> > > > **Molly Turner**
> > > > *Federal Grants Coordinator*
> > > > *EcoHealth Alliance Operations*

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--
Molly Turner
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[EXTERNAL] DEFUSE documents as submitted

Luke Hamel <hamel@ecohealthalliance.org>

Wed 3/28/2018 11:59 AM

To: Rocke, Tonie E <trocke@usgs.gov>
Cc: Dr. Peter Daszak <daszak@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>

Hi Tonie,

Peter had asked me to send these files to you. They are the final versions of our DEFUSE proposal, as submitted yesterday.

Attached files include:

- Technical and Management Proposal (Vol. I)
- Executive Summary Slide
- NWHC budget packet
- NWHC budget justification

Best,

Luke Hamel
Program Assistant

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(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

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(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

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(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)

[EXTERNAL] RE: DEFUSE documents as submitted

Peter Daszak <daszak@ecohealthalliance.org>

Wed 3/28/2018 12:07 PM

To: Luke Hamel <hamel@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>
Cc: Alison Andre <andre@ecohealthalliance.org>; Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>

..also want to add my thanks for your help getting this together Tonie!

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4474
www.ecohealthalliance.org
@PeterDaszak
@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Luke Hamel [mailto:hamel@ecohealthalliance.org]
Sent: Wednesday, March 28, 2018 1:00 PM
To: Rocke, Tonie
Cc: Peter Daszak; Alison Andre; Rachel Abbott; Richgels, Katherine
Subject: DEFUSE documents as submitted

Hi Tonie,

Peter had asked me to send these files to you. They are the final versions of our DEFUSE proposal, as submitted yesterday.

Attached files include:

- Technical and Management Proposal (Vol. I)
- Executive Summary Slide
- NWHC budget packet
- NWHC budget justification

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

[EXTERNAL] Re: DEFUSE documents as submitted

Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>

Wed 3/28/2018 2:34 PM

To: daszak@ecohealthalliance.org <daszak@ecohealthalliance.org>; hamel@ecohealthalliance.org <hamel@ecohealthalliance.org>
Cc: andre@ecohealthalliance.org <andre@ecohealthalliance.org>; Kateri.Paul@parc.com <Kateri.Paul@parc.com>; Rocke, Tonie E <trocke@usgs.gov>

Peter, Luke and the entire EHA team,

Thanks for all your work on this proposal too. We appreciate you bringing us on-board at the last minute. Looking forward to potentially doing great work with all of you. As we become more familiar with our different institutional capabilities, I'd also like for us to also explore other ways of working together.

Best,

Jerome

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Wednesday, March 28, 2018 at 10:28 AM
To: Luke Hamel <hamel@ecohealthalliance.org>, "Unidad, Jerome <Jerome.Unidad@parc.com>" <Jerome.Unidad@parc.com>
Cc: Alison Andre <andre@ecohealthalliance.org>, "Paul, Kateri <Kateri.Paul@parc.com>" <Kateri.Paul@parc.com>
Subject: RE: DEFUSE documents as submitted

And I'd like to add my thanks for helping us bring together a strong proposal, in rapid time!

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4474
www.ecohealthalliance.org
@PeterDaszak
@EcoHealthNYC

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conservation.

From: Luke Hamel [mailto:hamel@ecohealthalliance.org]
Sent: Wednesday, March 28, 2018 1:00 PM
To: Jerome.Unidad@parc.com
Cc: Peter Daszak; Alison Andre; Kateri.Paul@parc.com
Subject: DEFUSE documents as submitted

Hi Jerome,

Peter had asked me to send these files to you. They are the final versions of our DEFUSE proposal, as submitted yesterday.

Attached files include:

- Technical and Management Proposal (Vol. I)
- Executive Summary Slide
- PARC budget packet
- PARC budget justification

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

Sent Ite... received:2018-02-01..2018-03-31 Meet N RE

Print Cancel

Re: [EXTERNAL] DEFUSE proposal: fringe rate for National Wildlife Health Center?

Rocke, Tonie E <trocke@usgs.gov>

Tue 3/27/2018 10:25 AM

To: Molly Turner <turner@ecohealthalliance.org>
Cc: Anna Willoughby <willoughby@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>

Here are the documents and the email I sent yesterday to Luke, etc. Please note the budget total is slightly different due to the mistakes I found in the travel document that was originally provided to me.

Hello Luke: Attached is my budget justification (word and updated excel files) and my final budget. I found several mistakes in the original budget (miscalculations in the travel budget) which I fixed and also when time was added to my salary, the fringe was not adjusted. Thus the budget is slightly different but not by much. I think I have caught everything and it all adds up

Budget Justification Template (Please follow the guidance in this template for each budget period)

PHASE 1

BASE PERIOD 1

A. Personnel ($94,967)

- **Tonie E. Rocke**, **PhD**, Research Microbiologist, will oversee all aspects of developing methods to deliver immunomodulatory and immunoboosting agents to bats. Dr. Rocke has in-depth knowledge and experience in developing oral vaccines for use in controlling disease in wild animals, with particular expertise in developing vaccines for bats. We request $9,179 in salary for Dr. Tonie Rocke, who will dedicate 0.85 months p.a. to this project for phase1, year 1. An additional 1 month of Dr. Rocke's time will be provided in-kind.
- **Dr. Rachel Abbot, DVM, MS, Research Associate**, will help coordinate all field and laboratory activities as well as data compilation and analyses. Dr. Abbott has considerable experience in managing several previous vaccine projects in wild rodents and bats. We request $61,006 in salary for Dr. Abbott who will dedicate 12 months p.a. on this project for phase 1 year 1.

- **Undergraduate student assistants:** Three students will be hired for 3 to assist with bat feeding and husbandry for a total of $24,782.

B. Fringe ($18,445)

Fringe benefits are calculated as per US Geological Survey federally negotiated rate of 30.84% of base salary per year for Dr. Rocke and 26.35% for Dr. Abbott. Students receive an hourly wage but no fringe benefits

C. Travel ($9707.25)

Domestic MeetingTravel ($4,007,25): We are requesting $1,011.25 in phase 1, year 1 to support domestic travel from Madison, WI to Arlington, VA for one (1) Co-PI/Co-I to attend the DARPA Kickoff Meeting. We calculate the expenses per person as follows: 1 economy, round-trip tickets (Madison, WI <> Arlington Virginia) at $333, 2 nights in hotel at 437.50, plus $120 for parking and taxi and a total per diem allowance of $120.75.

We are requesting $2,996 in the phase 1, year 1 to support domestic travel from Madison, WI, to New York, New York for one (1) Co-PI/Co-I and one (1) research scientist to attend the annual meeting. We calculate the expenses per person as follows: 2 economy, round-trip tickets (Madison, WI <> New York,

New York) at $333/person, 3 nights in hotel at $291/person, and a total per diem allowance of $222/person, plus $140 for parking and taxi fare.

Domestic Field visit ($2316): We are requesting $2,316 in phase 1 year 1 to support domestic travel form Madison, Wisconsin to a cave site in Upper Peninsula, Michigan for 3 people (1 co-PI, 2 technicians) to test delivery methods in bats. We calculate the expenses per person as follows: 4 nights in a hotel at $93/person, a total per diem allowance of $204/person, and gas and use of government vehicle at a cost $588.

International Field visit ($3384)

We have budgeted for one Co-PI to travel for a cave site visit with local partners in China in phase 1, year 1. Expenses are calculated as follows: 1 economy round trip ticket (Madison, WI<> Kunming, Yunnan, China) for $1370, $1029 for lodging at $147 x 7 nights, a total per diem allowance of $805, plus $180 for parking and taxi fare for each visit.

E. Supplies and Materials (21,982.52)

Expenses are calculated as follows:

Animal Handling supplies ($11,288.67 Total): We are requesting $11,288.67 for phase 1, Year 1 for bat handling and supplies for both field and laboratory studies to include: a Harp trap for collecting bats, bat caging materials, mealworms for feeding bats, bat wing bands, anti-parasite medications, and PPE for handling bats (Cut resistant gloves, Tyvek suits, Tyvek aprons, N95 respirators, and PAPR replacement covers).

- **Vaccine production and biological sampling supplies ($10,693.85 Total)** We are requesting $10,693.85 for phase 1, year 1 to purchase necessary supplies for vaccine production and biological sampling including cell culture flasks, Nunc cell factories, fetal bovine serum, DMEM medium, glycerin jelly, rhodamine B, hair collection bags, 96 well plates, pipette tips and other consumables

F. Equipment (none requested)

G. Other direct costs

Animal Care ($12,600 Total) We are requesting $12,600 for phase 1, year 1. This amount covers animal care and room costs for up to 60 bats for 120 days at $105/day in a BSL3 animal facility, including daily husbandry, gut-loading meal worms, cleaning cages, veterinary services and daily surcharge for room use.

Rabies prophylaxis shots ($4020) We are requesting $4020 for phase 1, year 1 to provide rabies prophylactic vaccination for 4 animal care staff/year. Rabies vaccination is required of all staff handling bats due to the high prevalence of rabies in bats.

H. Indirect Costs ($Total)

We are requesting a federally negotiated indirect cost rate of 64.54% on all direct costs.

PHASE 1

BASE PERIOD 2

A. Personnel ($92,267)

- **Tonie E. Rocke**, **PhD**, Research Microbiologist, will oversee all aspects of developing methods to deliver immunomodulatory and immunoboosting agents to bats. Dr. Rocke has in-depth knowledge and experience in developing oral vaccines for use in controlling disease in wild animals, with particular expertise in developing vaccines for bats. We request $6,479 salary for Dr. Tonie Rocke, who will dedicate 0.6 months to this project for phase1, year 2. An additional 1 month of Dr. Rocke's time will be provided in-kind.
- **Dr. Rachel Abbot, DVM, MS, Research Associate**, will help coordinate all field and laboratory activities as well as data compilation and analyses. Dr. Abbott has considerable experience in managing several previous vaccine projects in wild rodents and bats. We request $61,006 in salary for Dr. Abbott who will dedicate 12 months on this project for phase 1 year 2.
- **Undergraduate student assistants:** Three students will be hired for 3 to assist with bat feeding and husbandry for a total of $24,782.

B. Fringe ($17,968)

Fringe benefits are calculated as per US Geological Survey federally negotiated rate of 30.84% of base salary per year for Dr. Rocke and 26.35% for Dr. Abbott. Students receive an hourly wage but no fringe benefits.

C. Travel ($10,561)

Domestic Field visit ($2316): We are requesting $2,316 in phase 1 year 1 to support domestic travel form Madison, Wisconsin to a cave site in Upper Peninsula, Michigan for 3 people (1 co-PI, 2 technicians) to test delivery methods in bats. We calculate the expenses per person as follows: 4 nights in a hotel at $93/person, a total per diem allowance of $204/person, and gas and use of government vehicle at a cost $588.

International Travel ($8245.50): We request $8,245.50 p.a. for phase 1, year 2 to support international travel from Madison, WI location to Annual Meeting in Wuhan, China. We calculate the expenses as follows: 1 economy, round trip ticket (Madison, WI<>Wuhan, China at $6,861, 5 nights hotel at $139.65, and a total per diem allowance of 546.25, plus $140 for parking and taxi fare.

E. Supplies and Materials (17,976.52)

Expenses are calculated as follows:

Animal Handling supplies ($7,282.67 Total): We are requesting $7,282.67 for phase 1, year 2 for bat handling and supplies for both field and laboratory studies to include bat caging materials, mealworms for feeding bats, bat wing bands, anti-parasite medications, and PPE for handling bats (Cut resistant gloves, Tyvek suits, Tyvek aprons, N95 respirators, and PAPR replacement covers).

- **Vaccine production and biological sampling supplies ($10,693.85 Total)** We are requesting $19,311.85 for phase 1, year 1-2, and phase 2, year 3 to purchase necessary supplies for vaccine production and biological sampling including cell culture flasks, Nunc cell factories, fetal bovine serum, DMEM medium, glycerin jelly, rhodamine B, hair collection bags, 96 well plates, pipette tips and other consumables

F. Equipment (none requested)

G. Other direct costs

Animal Care ($12,600 Total) We are requesting $12,600 for phase 1, year 1. This amount covers animal care and room costs for up to 60 bats for 120 days at $105/day in a BSL3 animal facility, including daily husbandry, gut-loading meal worms, cleaning cages, veterinary services and daily surcharge for room use.

Rabies prophylaxis shots ($4020) We are requesting $4020 for phase 1, year 1 to provide rabies prophylactic vaccination for 4 animal care staff/year. Rabies vaccination is required of all staff handling bats due to the high prevalence of rabies in bats.

H. Indirect Costs ($Total)

We are requesting a federally negotiated indirect cost rate of 64.54% on all direct costs.

PHASE 2

OPTION PERIOD 1

A. Personnel ($94,427)

- **Tonie E. Rocke**, **PhD**, Research Microbiologist, will oversee all aspects of developing methods to deliver immunomodulatory and immunoboosting agents to bats. Dr. Rocke has in-depth knowledge and experience in developing oral vaccines for use in controlling disease in wild animals, with particular expertise in developing vaccines for bats. We request $8,639 in salary for

Dr. Tonie Rocke, who will dedicate 0.8 months p.a. to this project for phase 2, option year 1. An additional 1 month of Dr. Rocke's time will be provided in-kind.

- **Dr. Rachel Abbot, DVM, MS, Research Associate**, will help coordinate all field and laboratory activities as well as data compilation and analyses. Dr. Abbott has considerable experience in managing several previous vaccine projects in wild rodents and bats. We request $61,006 in salary for Dr. Abbott who will dedicate 12 months p.a. on this project for phase 2, Option year 1

- **Undergraduate student assistants:** Three students will be hired for 3 to assist with bat feeding and husbandry for a total of $24,782.

B. Fringe ($18,634)

Fringe benefits are calculated as per US Geological Survey federally negotiated rate of 30.84% of base salary per year for Dr. Rocke and 26.35% for Dr. Abbott. Students receive an hourly wage but no fringe benefits

C. Travel ($11,430)

Domestic MeetingTravel ($2996):

We are requesting $2,996 in the phase 2, option year 1 to support domestic travel from Madison, WI, to New York, New York for one (1) Co-PI/Co-I and one (1) research scientist to attend the annual meeting. We calculate the expenses per person as follows: 2 economy, round-trip tickets (Madison, WI <> New York, New York) at $333/person, 3 nights in hotel at $291/person, and a total per diem allowance of $222/person, plus $140 for parking and taxi fare.

Domestic Field visit ($2316): We are requesting $2,316 in phase 2, option year 1 to support domestic travel from Madison, Wisconsin to a cave site in Upper Peninsula, Michigan for 3 people (1 co-PI, 2 technicians) to test delivery methods in bats. We calculate the expenses per person as follows: 4 nights in a hotel at $93/person, a total per diem allowance of $204/person, and gas and use of government vehicle at a cost $588.

International Field visit ($6118)

We have budgeted for one Co-PI and one research associate to travel for a cave site visit with local partners in China in phase 2, option year1. Expenses per person are calculated as follows: 1 economy round trip ticket (Madison, WI<> Kunming, Yunnan, China) for $1370, $1029 for lodging at $147 x 7 nights, a total per diem allowance of $805. An additional $180 is requested for parking and taxi fare.

E. Supplies and Materials ($17,976.52)

Expenses are calculated as follows:

Animal Handling supplies ($7,282.67 Total): We are requesting $7,282.67 for phase 2, option year 1 for bat handling and supplies for both field and laboratory studies to include: bat caging materials, mealworms for feeding bats, bat wing bands, anti-parasite medications, and PPE for handling bats (Cut resistant gloves, Tyvek suits, Tyvek aprons, N95 respirators, and PAPR replacement covers).

- **Vaccine production and biological sampling supplies ($10,693.85 Total)** We are requesting $10,693.85 for phase 2, option year 1 to purchase necessary supplies for vaccine production and biological sampling including cell culture flasks, Nunc cell factories, fetal bovine serum, DMEM medium, glycerin jelly, rhodamine B, hair collection bags, 96 well plates, pipette tips and other consumables

F. Equipment (none requested)

G. Other direct costs

Animal Care ($12,600 Total) We are requesting $12,600 for phase 2, option year 1. This amount covers animal care and room costs for up to 60 bats for 120 days at $105/day in a BSL3 animal facility, including daily husbandry, gut-loading meal worms, cleaning cages, veterinary services and daily surcharge for room use.

Rabies prophylaxis shots ($4020) We are requesting $4020 for phase 2, option year 1 to provide rabies prophylactic vaccination for 4 animal care staff/year. Rabies vaccination is required of all staff handling bats due to the high prevalence of rabies in bats.

H. Indirect Costs ($Total)

We are requesting a federally negotiated indirect cost rate of 64.54% on all direct costs.

PHASE 2

OPTION PERIOD 2

A. Personnel ($34,821)

- **Tonie E. Rocke**, **PhD**, Research Microbiologist, will oversee all aspects of developing methods to deliver immunomodulatory and immunoboosting agents to bats. Dr. Rocke has in-depth knowledge and experience in developing oral vaccines for use in controlling disease in wild animals, with particular expertise in developing vaccines for bats. We request $9,179 in salary for Dr. Tonie Rocke, who will dedicate 0.4 months to this project for phase 2, option period 2.
- **Dr. Rachel Abbot, DVM, MS, Research Associate**, will help coordinate all field and laboratory activities as well as data compilation and analyses. Dr. Abbott has considerable experience in

managing several previous vaccine projects in wild rodents and bats. We request $61,006 in salary for Dr. Abbott who will dedicate 6 months. on this project for phase 2, option period 2.

B. Fringe ($9,318)

Fringe benefits are calculated as per US Geological Survey federally negotiated rate of 30.84% of base salary per year for Dr. Rocke and 26.35% for Dr. Abbott.

C. Travel ($3501)

Domestic MeetingTravel ($3501):

We are requesting $3,501 in the phase 2, optional period 2 to support domestic travel from Madison, WI, to New York, New York for one (1) Co-PI/Co-I and one (1) research scientist to attend the annual meeting. We calculate the expenses per person as follows: 2 economy, round-trip tickets (Madison, WI <> New York, New York) at $333/person, 4 nights in hotel at $291 and a total per diem allowance of $296, plus $140 for parking and taxi fare for Co-PI and 3 nights in a hotel at $291 for research associate, total per diem allowance of $222 and $140 for parking and taxi fare.

E. Supplies and Materials ($2,163.43)

Expenses are calculated as follows:

Biological sampling supplies ($2,163,43 Total) We are requesting $2,163,43 for phase 2, option period 2 to purchase supplies to finish up biological sampling, including 96 well plates, pipette tips and other consumables.

F. Equipment (none requested)

TRAVEL

Trip #:	1	Location:	Arlington, VA, USA
Purpose:	DARPA Kickoff Meeting		

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem
1.75	1	$333.00	$69.00	$250.00

Itemized Expenses for "Other"

Description	Amount
Parking	$20.00
Transportation to/from airport and in Arlington	$100.00
Total:	$120.00

Trip #:	2	Location:	Kunming, Yunnan, China
Purpose:	China Cave Site Visit		

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem
7	1	$1,370.00	$115.00	$147.00

Itemized Expenses for "Other"

Description	Amount
Parking	$80.00
Transportation to/from airport and in Arlington	$100.00
Total:	$180.00

Trip #:	3	Location:	Upper Peninsula Michagan
Purpose:	US Cave Site Visit		

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem
4	3	$0.00	$51.00	$93.00

Itemized Expenses for "Other"

Description	Amount
Gas	$120.00
Government Car Use	$468.00
Total:	$588.00

Trip #:	4	Location:	New York, NY, USA
Purpose:	Annual Meeting (Rocke + Abbott)		

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem
3	2	$333.00	$74.00	$291.00

Itemized Expenses for "Other"

Description	Amount
Parking	$40.00
Transportation to/from airport and in New York	$100.00
Total:	$140.00

Trip #:	5	Location:	Upper Peninsula, Michigan, USA
Purpose:	US Cave Site Visit		

Days	# of People	Airfare	Meals & Incidental per diem	Lodging per diem
4	3	$0.00	$51.00	$93.00

Itemized Expenses for "Other"

Description	Amount
Gas	$120.00
Government Car Use	$468.00

	Total:	$588.00		
Trip #:	6	**Location:**	Wuhan, China	
Purpose:	Annual Meeting (Rocke)			
Days	**# of People**	**Airfare**	**Meals & Incidental per diem**	**Lodging per diem**
4.75	1	$6,861.00	$115.00	$147.00

Itemized Expenses for "Other"

Description	**Amount**
Parking	$40.00
Transportation to/from airport in Wuhan	$100.00
Total:	$140.00

Trip #:	7	**Location:**	Upper Peninsula, Michigan, USA	
Purpose:	US Cave Site Visit			
Days	**# of People**	**Airfare**	**Meals & Incidental per diem**	**Lodging per diem**
4	3	$0.00	$51.00	$93.00

Itemized Expenses for "Other"

Description	**Amount**
Gas	$120.00
Government Car Use	$468.00
Total:	$588.00

Trip #:	8	**Location:**	Kunming, Yunnan, China	
Purpose:	Deployment Visit			
Days	**# of People**	**Airfare**	**Meals & Incidental per diem**	**Lodging per diem**
7	2	$1,370.00	$115.00	$147.00

Itemized Expenses for "Other"

Description	**Amount**
Parking	$80.00
Transportation to/from airport and in Kunming	$100.00
Total:	$180.00

Trip #:	9	**Location:**	New York, NY, USA	
Purpose:	Annual Meeting (Rocke + Abbott)			
Days	**# of People**	**Airfare**	**Meals & Incidental per diem**	**Lodging per diem**
3	2	$666.00	$74.00	$291.00

Itemized Expenses for "Other"

Description	**Amount**
Parking	$40.00
Transportation to/from airport	$100.00
Total:	$140.00

Trip #:	10	**Location:**	New York, NY, USA	
Purpose:	Annual Meeting (Rocke + Abbott)			
Days	**# of People**	**Airfare**	**Meals & Incidental per diem**	**Lodging per diem**
4	1	$333.00	$74.00	$291.00
3	1	$333.00	$74.00	$291.00

Itemized Expenses for "Other"

Description	**Amount**
Parking	$40.00
Transportation to/from airport and in New York	$100.00

	Total:	$140.00

Contract Period
Base 1

Other	Total
$120.00	$1,011.25

Contract Period
Base 1

Other	Total
$180.00	$3,384.00

Contract Period
Base 1

Other	Total
$588.00	$2,316.00

Contract Period
Base 1

Other	Total
$140.00	$2,996.00

Contract Period
Base 2

Other	Total
$588.00	$2,316.00

Contract Period
Base 2

Other	Total
$140.00	$8,245.50

Contract Period
Option I

Other	Total
$588.00	$2,316.00

Contract Period
Option I

Other	Total
$180.00	$6,588.00

Contract Period
Option I

Other	Total
$140.00	$2,996.00

Contract Period
Option II

Other	Total
$140.00	$1,933.00
$140.00	$1,568.00

MATERIALS/

Item	Manufacturer	Part Number	Unit Price	Quantity
Harp Trap	Bat conservation and management		$2,003	2
Mealworms	Rainbow mealworms		$100/20,000	12
bat caging materials	various		$500/cage	9
bat wing bands	Porzana		$596/box	9
Cut resistant gloves	Varied		$15/pr	30
Tyvek suits	DuPOnt	EV29135313	$306/case	15
Tyvek aprons	Lakeland	6EHH7	$58/case	15
N95 respirators	3M	9511	$20/box	45
PAPRs replacement covers	3M		$96/3 units	45
Selamectin	Zoetis		$250	
cell culture flasks	Corning	430641U	415/case	5
cell culture flasks	Corning	431080	425/case	10
Nunc cell factories	Nunc	140250	$370/case	12
fetal bovine serum	GE Hyclone	SH30071.03	$600/bottle	8
DMEM medium	GE Hyclone	SH30021.02	$30/l	10
glycerin jelly	Carolina Biological Supply		$43 bottle	50
rhodamine B	Sigma		$56/100g	6
hair collection bags	U-line		$75/box	10
96 well plates	Corning	3599	$600/case	8
pipette tips	Fisher	13-676-10	$100/case	50
Consumables	miscellaneous			
				Total

Y1 Total

Y2 Total

Y3 Total

Y3.5 Total

EQUIPMENT

Total Price	Contract Period	Additional Information
$4,006.00	Y1	
$1,200.00	Y1-Y3	
$4,500.00	Y1-Y3	custom made
$4,768.00	Y1-Y3	
$450.00	Y1-Y3	
$4,590.00	Y1-Y3	
$870.00	Y1-Y3	
$900.00	Y1-Y3	
$4,320.00	Y1-Y3	
$250.00	Y1-Y3	
$2,075.00	Y1-Y3	
$4,250.00	Y1-Y3	
$4,440.00	Y1-Y3	
$4,800.00	Y1-Y3	
$300.00	Y1-Y3	
$2,150.00	Y1-Y3	
$336.00	Y1-Y3	
$750.00	Y1-Y3	
$4,800.00	Y1-Y3.5	
$5,000.00	Y1-Y3.5	
$5,344.00	Y1-Y3.5	needles, syringes,whirl paks, plastic bags, other disposables, all <5K
$60,099.00		

$25,854.00

$21,982.52

$17,976.52

$17,976.52

$2,163.43

$60,098.99

OTHER DI

Description	Total Price	Contract Period
animal perdiem costs	$12,600	Base 1
animal perdiem costs	$12,600	Base 2
animal perdiem costs	$12,600	Option 1
rabies prphylactic shots	$4,020	Base 1
rabies prphylactic shots	$4,020	Base 2
rabies prphylactic shots	$4,020	Option 1
Total	$49,860	

[RECT COSTS

Additional Information
up to 60 bats for 120 days at $105/day in BSL3 animal facility, includes daily husbandry, gut-loading meal worms, cleaning cages, feeding bats, veterinary services and daily surcharge for rom use,
up to 60 bats for 120 days at $105/day in BSL3 animal facility (ame as above)
up to 60 bats for 120 days at $105/day in BSL3 animal facility (same as above)
all animal care and technical staff must be vaccinated against rabies to work with bats. 1005/person
all animal care and technical staff must be vaccinated against rabies to work with bats. 1005/person
all animal care and technical staff must be vaccinated against rabies to work with bats. 1005/person

WORKSPACE FORM

1-800-518-4726
SUPPORT@GRANTS.GOV

This Workspace form is one of the forms you need to complete prior to submitting your Application Package. This form can be completed in its entirety offline using Adobe Reader. You can save your form by clicking the "Save" button and see any errors by clicking the “Check For Errors” button. In-progress and completed forms can be uploaded at any time to Grants.gov using the Workspace feature.

When you open a form, required fields are highlighted in yellow with a red border. Optional fields and completed fields are displayed in white. If you enter invalid or incomplete information in a field, you will receive an error message. Additional instructions and FAQs about the Application Package can be found in the Grants.gov Applicants tab.

OPPORTUNITY & PACKAGE DETAILS:

Opportunity Number:	HR001118S0017
Opportunity Title:	PREventing EMerging Pathogenic Threats
Opportunity Package ID:	PKG00237724
CFDA Number:	12.910
CFDA Description:	Research and Technology Development
Competition ID:	
Competition Title:	
Opening Date:	01/19/2018
Closing Date:	03/27/2018
Agency:	DARPA - Biological Technologies Office
Contact Information:	BAA Coordinator PREEMPT@darpa.mil

APPLICANT & WORKSPACE DETAILS:

Workspace ID:	WS00094394
Application Filing Name:	Project DEFUSE
DUNS:	0770900660000
Organization:	ECOHEALTH ALLIANCE INC.
Form Name:	R & R Subaward Budget 10 YR Subform
Form Version:	1.4
Subform Name:	USGS Ntl. Wildlife Health Cen
Requirement:	Optional
Download Date/Time:	Mar 06, 2018 05:28:38 PM EST
Form State:	Error(s)

FORM ACTIONS:

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 1** **Start Date:** 12/01/2018 **End Date:** 11/30/2019

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.85			9,179.00	2,475.00	11,654.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 88,630.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00	0.00	24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel 24,782.00

Total Salary, Wages and Fringe Benefits (A+B) 113,412.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	6,323.25
2. Foreign Travel Costs	3,384.00
Total Travel Cost	9,707.25

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	21,982.52
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.	Rabies prophylaxis	4,020.00
10.		
	Total Other Direct Costs	38,602.52

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	161,721.77

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	161,721.77	104,375.23

Total Indirect Costs 104,375.23

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	266,097.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	266,097.00

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 2** **Start Date:** 12/01/2019 **End Date:** 11/30/2020

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.60			6,479.00	1,998.00	8,477.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 85,453.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00	0.00	24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel: 24,782.00

Total Salary, Wages and Fringe Benefits (A+B): 110,235.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	2,316.00
2. Foreign Travel Costs	8,245.50
Total Travel Cost	10,561.50

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	17,976.52
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.	Rabies prophylaxis	4,020.00
10.		
	Total Other Direct Costs	34,596.52

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	155,393.02

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	155,393.02	100,290.65

Total Indirect Costs 100,290.65

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	255,683.67

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	255,683.67

L. Budget Justification

(Only attach one file.) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 3** **Start Date:** 12/01/2020 **End Date:** 11/30/2021

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.80			8,639.00	2,664.00	11,303.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 88,279.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00	0.00	24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel 24,782.00

Total Salary, Wages and Fringe Benefits (A+B) 113,061.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	5,312.00
2. Foreign Travel Costs	6,118.00
Total Travel Cost	11,430.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	17,976.52
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.	Rabies prophylaxis	4,020.00
10.		
	Total Other Direct Costs	34,596.52

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	159,087.52

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	158,087.52	102,029.68

Total Indirect Costs 102,029.68

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	261,117.20

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	261,117.20

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 4

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 4** **Start Date:** 12/01/2021 **End Date:** 03/31/2022

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.40			4,319.00	1,332.00	5,651.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	6.00			30,502.00	7,986.00	38,488.00
Project Role:	Associate Scientist										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 44,139.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						

Total Number Other Personnel

Total Other Personnel

Total Salary, Wages and Fringe Benefits (A+B) 44,139.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	3,501.00
2. Foreign Travel Costs	
Total Travel Cost	3,501.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	2,163.43
2.	Publication Costs	6,000.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.		
9.		
10.		
	Total Other Direct Costs	8,163.43

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	55,803.43

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	55,803.43	36,015.26

Total Indirect Costs 36,015.26

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	91,818.69

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	91,818.69

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		306,501.00
Section B, Other Personnel		74,346.00
Total Number Other Personnel	9	
Total Salary, Wages and Fringe Benefits (A+B)		380,847.00
Section C, Equipment		
Section D, Travel		35,199.75
1. Domestic	17,452.25	
2. Foreign	17,747.50	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		115,958.99
1. Materials and Supplies	60,098.99	
2. Publication Costs	6,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	37,800.00	
9. Other 2	12,060.00	
10. Other 3		
Section G, Direct Costs (A thru F)		532,005.74
Section H, Indirect Costs		342,710.82
Section I, Total Direct and Indirect Costs (G + H)		874,716.56
Section J, Fee		
Section K, Total Costs and Fee (I + J)		874,716.56

Re: [EXTERNAL] PREEMPT - A few important items

Rocke, Tonie E <trocke@usgs.gov>

Mon 3/26/2018 3:54 PM

To: Luke Hamel <hamel@ecohealthalliance.org>
Cc: Daszak Peter <daszak@ecohealthalliance.org>; Tonie Rocke <(b) (6) @gmail.com>; Abbott, Rachel (Contractor) C <rabbott@contractor.usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>; Jonathon Musser <musser@ecohealthalliance.org>; Evelyn Luciano <luciano@ecohealthalliance.org>

Hi Luke: Attached are my comments on the full draft; I added my comments to Jerome's draft. I corrected a few errors, deleted at least one sentence in my section, and also added my deliverables for Task 7. I'm not certain what you want for project metrics. A timeline or something? Just repeating the deliverables doesn't seem appropriate. Also, I have someone checking on the CAGE code. Let me know if you need anything else. Thanks! -Tonie

On Mon, Mar 26, 2018 at 2:32 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Hi Tonie,
>
> Thank you for the facility description. There won't be a need for us to have a call, but please review the draft and look for ways to reduce the text of your technical section.
>
> Additionally, I was hoping to confirm the following two points:
>
> (1) Is NWHC's CAGE code the following? **52Y40**
>
> (2) Which of the following 'organization types', best describes NWHC?
>
> -"LARGE BUSINESS", "SMALL DISADVANTAGED BUSINESS", "OTHER SMALL BUSINESS", "HBCU", "MI", "OTHER EDUCATIONAL", OR "OTHER NONPROFIT";
>
> Best,
>
>
> **Luke Hamel**
> *Program Assistant*
>
> EcoHealth Alliance
> 460 West 34th Street – 17th floor
> New York, NY 10001
>
> (b) (6) (direct)
> (b) (6) (mobile)
> www.ecohealthalliance.org
>
>
> ***EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.***

On Mon, Mar 26, 2018 at 3:16 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

Hello again Luke: Here is a brief description of our facility. Let me know if this is sufficient. Also, will we be having a call or not? I have not yet had a chance to review the technical proposal but will do so shortly.

On Mon, Mar 26, 2018 at 2:01 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

Excellent. Thank you for all of your work on this, Tonie! I will speak with Jonathon and address your question regarding the DARPA kick-off meeting.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Mon, Mar 26, 2018 at 2:58 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

Hello Luke: Attached is my budget justification (word and updated excel files) and my final budget. I found several mistakes in the original budget (miscalculations in the travel budget) which I fixed and also when time was added to my salary, the fringe was not adjusted. Thus the budget is slightly different but not by much. I think I have caught everything and it all adds up now, but feel free to check. I will send facility description along soon. Thanks -Tonie

On Mon, Mar 26, 2018 at 8:28 AM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

Hi Tonie,

I hope you had a great trip. If you are able to begin drafting a budget justification, that would be very helpful. Whatever you cannot complete, we will be sure to get done.

Regarding the budget justification, I have reattached a template with appropriate headings and language that is already correctly formatted. **I would just ask you to insert the appropriate**

name/cost amount, substituting CAPITALIZED words and filling in gaps (indicated by underscores).

Each section in the budget (e.g. Personnel, fringe, travel, etc.) should have a corresponding section in the budget justification (as shown in the template). Essentially, any line item that is listed in the budget needs to be justified in the 'budget justification' document.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Mon, Mar 26, 2018 at 8:00 AM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Luke: I have returned from Mexico and just wading through email. Do you still need me to prepare a budget justification in a word document (everything was in the excel file) this AM? I'll get on it right away if it hasn't already been done. Please advise. Best -Tonie
>
> On Sat, Mar 24, 2018 at 1:29 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > It looks as though we have a detailed budget from you, but we still will need a budget justification (essentially a Word doc. that provides justification for each line item of the budget).
> >
> > Rachel and Katie - If you have time this weekend to get a start on the budget justification doc, that would be very helpful. If you're not available, which I understand may very well be the case, we will be happy to take this on. Please let us know.
> >
> > Thank you,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Fri, Mar 23, 2018 at 4:46 PM, Tonie Rocke <(b) (6) @gmail.com> wrote:

> Hello all: I sent my budget justification to Ana several days ago. Thanks for addressing safety issues Rachel!
>
> Sent from my iPhone
>
> On Mar 23, 2018, at 2:38 PM, Abbott, Rachel <rabbott@usgs.gov> wrote:
>
> > Hi Luke,
> > I have added some paragraphs to the page you sent. It just deals with safety of RCN, so I hope that is enough. Most of the text came out of documents we have to write to get approval to use our RCN vaccines in the field (risk analysis for USDA CVB and environmental assessment for USGS). Unfortunately, as I said before, I'll be unavailable until next Thursday, but Tonie should be back in her office on Monday.
> > --Rachel
> >
> > On Fri, Mar 23, 2018 at 1:45 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
> >
> > Hi Rachel and Katherine,
> >
> > I wanted to address a few important PREEMPT items with you:
> >
> > - Regarding **NWHC budget justification**
> > - If you have not already done so (and I apologize for not knowing the answer), could you please send us your budget justification document? We are hoping to have all collaborator budgets and budget justifications as soon as possible.
> > - Regarding language on **'Long-term safety and efficacy'**
> > - In the PREEMPT proposal, we must state how we will establish methods to assess the 'long-term safety and efficacy of our preemptive approaches'

    - Given your field of work, do you have any existing language on how to address potential negative impacts of intervention approaches on non-target species?
    - I have attached language from the BAA to provide you with further guidance on what DARPA requires us to include.
    - This being said - Rachel and Katherine, could you please write-up a short section (a paragraph or so), that addresses this issue 'long-term safety and efficacy'?
        - I apologize for the extremely short notice, but **we would greatly appreciate it if you could return this to us by tomorrow afternoon, Sat. (3/24).**
- Regarding '**pricing assumptions'** for NWHC facilities
    - Previously, we had asked you to identify any 'pricing assumptions' that may correspond with use of government facilities. Due to confusion about what exactly was being asked for, we reached out to DARPA staff, asking them to clarify the matter:
        - We asked: "EcoHealth Alliance has a USG entity listed as a subcontractor in our proposal. Is the USG entity required to identify any pricing assumptions beyond those within their fully detailed and documented budget?
        - To which they responded: "No"
            - Long story short...there is NO need for you to identify any additional pricing assumptions.

Thank you and please let me know if you have any questions. I will be available by email and phone (mobile number listed below) over the weekend, should you need to contact me.

Best,

Luke Hamel
Program Assistant

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EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

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A. EXECUTIVE SUMMARY

Technical Approach: Our goal is to defuse the potential for spillover of novel bat-origin high-zoonotic risk SARS-related coronaviruses in Southeast Asia. **In TA1** we will develop **host-pathogen ecological niche models** to predict the species composition of bat caves across Southeast Asia. We will parameterize this with a full inventory of host and virus distribution at our field sites, three caves in Yunnan Province, China and a series of unique datasets on bat host-viral relationships. By the end of Y1, we will use these to create a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens at any site across Asia. We will intensively sample bats at our field sites to sequence SARSr-CoV spike proteins, reverse engineer them to conduct binding assays, and insert them into SARS-CoV backbones to infect humanized mice to assess capacity to cause SARS-like disease. Our modeling team will use these data to build **machine-learning genotype-phenotype models** of viral evolution and spillover risk. We will uniquely validate these with human serology data through LIPS assays designed to assess which spike proteins allow spillover into people.

In TA2, we will evaluate two approaches to reduce SARSr-CoV shedding in cave bats: (**1) Broadscale Immune Boosting**, in which we will inoculate bats with immune modulators to upregulate their innate immune response and downregulate viral replication; **(2) Targeted Immune Priming**, in which we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance innate immunity against specific, high-risk viruses. We will trial inoculum delivery methods on captive bats including automated aerosolization, transdermal nanoparticle application and edible, adhesive gels. We will use stochastic simulation modeling informed by field and experimental data to characterize viral dynamics in our cave sites, to maximize timing, inoculation protocol, delivery method and efficacy of viral suppression. The most effective delivery method and treatments will be trialed in our experimental cave sites in Yunnan Province, with reduction in viral shedding as proof-of-concept.

Management Approach: Members of our collaborative group have worked together on bats and their viruses for over 15 years. The lead organization, EcoHealth Alliance, will oversee all modeling, lab, and fieldwork. EHA staff will develop models to evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for delivery of both immune boosting and immune targeting inocula. Specific work will be subcontracted to the following organizations:

- Prof. Ralph Baric, UNC, will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades.
- Prof. Linfa Wang, Duke-NUS, will lead work on immune boosting, building from his groups' pioneering work on bat immunity.

- Dr. Zhengli Shi, Wuhan Institute of Virology will conduct viral testing on all collected samples, binding assays and some humanized mouse work.
- Dr. Tonie Rocke, USGS National Wildlife Health Center will develop a delivery method for immunological countermeasures, following from her work on vaccine delivery in wildlife, including bats.
- Dr. Jerome Unidad, PARC will develop an innovative aerosol technology that could work with a wide-range of formulations into a field-deployable device that can be used for large-scale inoculation of bats.

B. EXECUTIVE SUMMARY SLIDE

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C. GOALS AND IMPACT

Overview

The overarching goals of DEFUSE are:

- **Identify and model the spillover risk** of novel SARS-related CoVs in South and SE Asia
- Design and demonstrate proof-of-concept that interventions to upregulate the naturally low innate immunity of bats to viruses (**immune boosting**) and to high risk SARSr-CoVs in particular (**immune priming**) will transiently reduce spillover risk.

We will analyze, design and field-test a novel strategy to reduce risk of viral emergence from bats that will help protect the warfighter within SACOM and SEACOM, and will be scalable to other systems including Ebola virus, rabies and other bat-origin pathogens.

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Innovation and uniqueness:

Bats harbor more emerging zoonoses than any other group of mammals, and are ubiquitous, abundant, wide-ranging and often overlooked. Despite this, other than PPE, there is no available current technology to reduce the risk of exposure to novel coronaviruses from bats. Models of bats' capacity to harbor viruses, of ecological and environmental drivers of their emergence, and of the evolutionary potential of different strains to spillover are rudimentary. No vaccines or therapeutics exist for SARSr-CoVs, and exposure mitigation strategies are non-existent. SARSr-CoVs are enzootic in Asian, African[1], and European bats[2] that roost in caves but forage widely at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have recently shown evidence of spillover of SARSr-CoVs into people in China, unrelated to the original SARS pandemic, and have isolated strains capable of producing SARS-like disease in humanized mice that don't respond to antibody treatment or vaccination. These viruses are a clear-and-present danger to our military and to global health security because of their continuous circulation and evolution in bats and periodic spillover into humans in locations where surveillance is virtually nonexistent.

Commented [PD2]: There's a new ref that I tweeted about recently - http://coronavirus.fr/publications/

EcoHealth Alliance leads the world in predictive models of viral emergence. We will build on our machine-learning models of spillover hotspots, host-pathogen ecological niche and genotype-phenotype mapping by incorporating unique datasets to validate and refine hotspot risk maps of viral emergence in SE Asia and beyond. We have shown that bats are able to carry otherwise lethal viruses by virtue of dampened innate immunity (e.g. inflammatory) pathways, which likely evolved as an adaptation to the physiologic stress of flight. We will use this insight to design strategies, like small molecule Rig-like receptor (RLR) or Toll-like receptor (TLR) agonists, to upregulate bat immunity and down-regulate viral replication in their cave roosts, thereby significantly reducing the frequency and magnitude of viral shedding and spillover **(broadscale immune boosting strategy)**. We will complement this by treating bats with novel chimeric polyvalent recombinant spike proteins to enhance their adaptive immune response against specific, high-risk coronaviruses **(targeted immune priming strategy)**, especially when their innate immune response is boosted as above. We will design novel automated application methods, based on our previous work delivering wildlife vaccines, to apply these interventions in a way that eliminates the need for a person to enter a cave and potentially get exposed to bat borne viruses or other hazards.

Technical Area 1

Our strategy to reduce spillover risk of bat SARS-related CoVs begins with modeling to predictively assess spillover risk across South and SE Asia using baseline genotype-phenotype analysis of host and strain diversity from the literature, from surveillance in our designated model caves in China, and across the region in other projects. In TA1, the DEFUSE modeling and analytics team, will build joint species distribution models (JSDM) of environmental and

ecological correlates and traits of cave bat communities to predict species composition of bat caves across Southern China, South and SE Asia. Dr. Epstein at EHA will coordinate animal experimental work with the teams at NWHC, Duke-NUS and Wuhan and radio telemetry studies with the field surveillance team. We will then use a series of datasets we have built to produce host-virus risk models for the region. These include our comprehensive database of bat host-viral relationships and estimates of zoonotic viral richness per bat species[3]; biological inventory data on all bat caves in Southern China; and modeled species distribution data for all bats. We will parameterize the model with data from three cave sites in Yunnan, China (one with high-risk SARSr-CoVs, two other control/comparison sites), including: radio- and GPS-telemetry to identify home range and additional roost sites for each bat species; inventory of bat population density, distribution and segregation and their daily, weekly and seasonal changes; viral prevalence and individual viral load; shedding of low- and high-risk SARSr-CoV strains among bat species, age classes, genders; and telemetry and mark-recapture data to assess metapopulation structure and inter-cave connectivity. We will test and validate model predictions of a cave's viral spillover potential with data from prior PREDICT sampling in 7 other Asian countries. At the end of Yr 1, we will produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens in a region. **The 'Spatial viral spillover risk' app** will be updated real-time with surveillance data (e.g. field-deployable iPhone and android compatible echolocation data) from our project and others, to ground-truth and fine-tune its predictive capacity.

The Wuhan Institute of Virology team will test bat fecal, oral, and blood samples for SARSr-CoVs. We will collect viral load data using fresh fecal pellets from individually sampled bats and from tarps laid on cave floors deployed where necessary to reduce roost disturbance. SARSr-CoV spike proteins will be sequenced, analyzed phylogenetically for recombination events, and high-risk viruses (spike proteins close to SARS-CoV) characterized and isolated. The UNC team will reverse-engineer spike proteins to conduct binding assay to human ACE2 (the SARS-CoV receptor). They will culture SARS-like bat coronaviruses to distinguish high-risk strains that can replicate in primary human cells and low risk strains that require exogenous enhancers. Viral spike glycoproteins that bind receptors will be inserted into SARS-CoV backbones, inoculated into human cells and humanized mice to assess capacity to cause SARS-like disease, and to be blocked by monoclonal therapies, the nucleoside analogue inhibitor GS-5734[4] or vaccines against SARS-CoV[4-8].

The EHA modeling team will use these data to **build models of risk of viral evolution and spillover**. These genotype-to-phenotype machine-learning models will predict viral ability to infect human host cells based on genetic traits and results of receptor binding and mouse infection assays. Using data on diversity of spike proteins, recombinant CoVs, and flow of genes within each bat cave via bat movement and migration, we will estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection.

Commented [3]: Are we saying only one cave site has SARSr-CoVs, or does one site have a higher prevalence of these compared to controls? Should validate this with our prelim data if possible. Are 3 sites sufficient?

Commented [4]: no need for urogenital samples and these bats are too small to collect those anyway. Fecal and oral are key. Blood is also important for serolgy

Commented [5]: Edited this slightly, but we could just go with individually sampled bats, should be easy enough to say we'll sample ~200 individually trapped bats on a monthly basis at cave entrances using harp trap, if we want to do away with tarp sampling. Alternatively we could leave both and justify the use of tarps and that we'll use high-resolution photos of bats roosting in caves to estimate population size from populations sampled non-invasively using tarp collection.

Finally, virus-host relationship and bat home range data will be used to estimate spillover potential - extending models well beyond our field sites. We will then **validate model predictions of viral spillover risk** by **1)** conducting spike protein-based binding and cell culture experiments, and **2)** identifying spillover strains in people near our bat cave sites. Our preliminary work on this shows ~3% seroprevalence to SARSr-CoVs, using a specific ELISA [REF]. We will design LIPS assays to the specific high- and low- zoonotic-risk SARSr-CoVs identified in this project as we have done previously [REF]. We will use previously collected and newly collected human sera from these populations to test for presence of antibodies to the high- and low-risk SARSr-CoVs identified by our modeling. We will then **model optimal strategies to maximize treatment efficacy** for TA2, using stochastic simulation modeling informed by field and experimental data to characterize viral circulation dynamics in bats. We will estimate frequency and population coverage required for our intervention approaches to suppress viral spillover. We will determine the seasons, locations within a cave, and delivery methods (spray, swab, or automated cave mouth or drone) that will be most effective. Finally we will determine the time period treatment will be effective for, until re-colonization or evolution leads to return of a high-risk SARSr-CoV.

Technical Area 2

In TA2, we will **develop scalable approaches that target and suppress the animal virus in its reservoir(s)and/or vector(s), to reduce the likelihood of virus transmission into humans**. We will evaluate two approaches to defuse SARS-related CoV spillover potential**: 1) Broadscale Immune Boosting:** using the unique immune damping in bats that our group has discovered, we will apply immune modulators like bat interferon to live bats, to up-regulate their naïve immunity and then assess their ability to suppress viral replication and shedding; **2) Targeted Immune Priming:** building on preliminary development of polyvalent chimeric recombinant SARSr-CoV spike proteins, we will conduct application trials with live bats to assess suppression of replication and shedding of a broad range of dangerous SARS-related CoVs.

Both lines of work will begin in Yr 1 and run parallel. Prof. Linfa Wang (Duke-NUS) will lead the immune boosting work, building on his pioneering work on bat immunity[9] which shows that the long-term coexistence of bats and their viruses has led to equilibrium between viral replication and host immunity. This is likely due to down-regulation of their innate immune system as a fitness cost of flight[9]. The weakened functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may allow bats to maintain an effective, but not over-response to viruses[10]. A similar finding was observed for bat IFNA, which is less abundant but constitutively expressed without stimulation[11]. Given high native SARSr-CoV load in bats, we aim to boost bat innate immunity through the IFN pathway, break the host-virus equilibrium to suppress bat SARSr-CoV replication and shedding.

We will trial the following, concurrently and competitively, for efficiency, cost and scalability: **i)** Universal bat interferon. Aerosol spraying or intranasal application of IFN or other small molecules reduces viral loads in humans, ferrets and mouse models[12,13]. Interferon has been used clinically when antiviral drugs are unavailable, e.g. against filoviruses[14]. Replication of SARSr-CoV is sensitive to interferon treatments, as shown in our previous work[13]; **ii)** Boosting bat IFN by blocking bat-specific IFN negative regulators. Uniquely, bat IFNA is naturally constitutively expressed but cannot be induced to a high level[11], indicating a negative regulatory factor in the bat interferon production pathway. We will use CRISPRi to identify the negative regulator and then screen for compounds targeting this gene; **iii)** Activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7-dependent pathways. Our work showing that mutant bat STING restores antiviral functionality suggests these pathways are important in bat-viral coexistence[10]. By identifying small molecules to directly activate downstream of STING, we will activate bat interferon and promote viral clearance. A similar strategy will be applied to ssRNA-TLR7-dependent pathways; **iv)** Activating functional bat IFN production pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been demonstrated in a mouse model for SARS-CoV, IAV and HBV[12,15]; **v)** Inoculating crude coronavirus fragments to upregulate innate immune responses to specific CoVs – a partial step towards the targeted immune priming work below.

Prof. Ralph Baric (UNC) will lead the immune priming work. He will develop recombinant chimeric spike-proteins[16]from our known SARSr-CoVs, and those we characterize during project DEFUSE. The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain[17]. In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles[6,18-21]. We will test these in controlled lab conditions, taking the best candidate forward for testing in the field. We have built recombinant spike glycoproteins harboring structurally defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broad scale immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, potentially reducing disease risk (and transmission risk to people) in these animals for longer periods[22,23].

The immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established the only experimental breeding colony of cave bats (*Eonycteris spelaea*) in SE Asia. This genus is evolutionarily related to *Rhinolophus* spp. (the hosts of SARSr-CoVs), so we have confidence that results will be transferable. Our initial proof-of-concept tests will be in this experimental colony, extended to a small group of wild-caught *Rhinolophus*

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sinicus bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with *Rhinolophus* sp. bats in the BSL-4 facility at CSIRO, AAHL (L.Wang, unpublished results).

Finally, work on a delivery method for our immune boosting and priming molecules will be developed and implemented by Dr. Tonie Rocke at the USGS, National Wildlife Health Center who has previously developed animal vaccines through to licensure[24]. Using locally acquired insectivorous bats[25,26], we will assess delivery vehicles and methods including: 1) transdermally applied nanoparticles; 2) series of sticky edible gels that bats will groom from themselves and each other; 3) aerosolization via sprayers that could be used in cave settings; 4) automated sprays triggered by timers and movement detectors at critical cave entry points, and 5) sprays delivered by remote controlled drone. We have already used simple gels to vaccinate bats against rabies in the lab[25], and hand delivered these containing biomarkers to vampire bats in Peru and Mexico to show they are readily consumed and transferred among bats. In our bat colony, we will trial delivery vehicles using the biomarker rhodamine B (which marks hair and whiskers upon consumption) to assess uptake. The most optimal approaches will then be tested on wild bats in our three cave sites in Yunnan Province with the most successful immunomodulators from TA2. Fieldwork will be conducted under the auspices of Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance). A small number of bats will be captured and assayed for viral load and immune function after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has had unique access to these sites for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for experimental trials from the Provincial Forestry Department. We expect to be successful, as we have worked with the Forestry Department collaboratively for 10 years, with support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife. EHA has a proven track record of rapidly obtaining IACUC and DoD ACURO approval for bat research.

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Deliverables:

- App identifying geographical risk of spillover for novel SARSr-CoVs in SE Asia
- Identified indicators (modeled and validated) of spillover capacity for different viral strains.
- Proven mechanistic approach to modulating bat innate immunity to reduce viral shedding
- Tested and validated delivery mechanism for bat cave usage including vaccines in other bat host-pathogen systems (e.g. rabies, WNS).
- Proof-of-concept approach to transiently reducing viral shedding in wild bats that can be adapted for other systems including Ebola virus.

D. TECHNICAL PLAN

Technical Area I:

Choice of site and model host-virus system. For the past 14 years, our team has conducted coronavirus surveillance in bat populations across Southern China, resulting in <150 CoV identifications in ~10,000 samples[27-29]. Bat SARSr-CoVs are genetically diverse, especially in the S gene, and most are highly divergent from SARS-CoV. However, in a cave site complex in Yunnan Province, we have found bat SARSr-CoVs with S genes extremely similar to SARS-CoV, and which, as a quasispecies population assemblage contain all the genetic components of epidemic SARS-CoV[30].

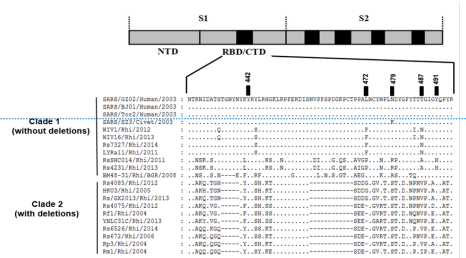

Fig. 1: Alignment of amino acid sequence of the receptor-binding motif in the spike protein of SARSr-CoVs and SARS-CoV[30]. Numbered amino acid is the key residues which is responsible for SARS-CoV S and human ACE2 interaction[31].

We have isolated three strains at this site (WIV1, WIV16 and SHC014) that unlike other SARSr-CoVs, do not contain two deletions in the receptor-binding domain (RBD) of the spike, and share substantially higher sequence identity to SARS-CoV (Fig. 1). These viruses have been demonstrated to use human ACE-2 receptor for cell entry as SARS-CoV does (Fig. 2), and replicate efficiently in various animal and human cells[27,29,30,32,33] including primary human lung airway cells, similar to epidemic SARS-CoV[7,8].

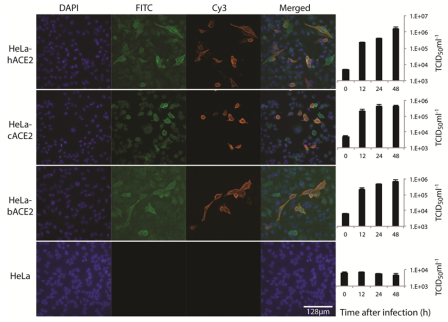

Fig. 2: Bat SARSr-CoV WIV1 replicates efficiently in HeLa cells expressing human, civet and bat ACE2[29].

Chimeras (recombinants) with these SARSr-CoV S genes inserted into a SARS-CoV backbone, as well as synthetically reconstructed full length SHCO14 and WIV-1 bat viruses cause SARS-like illness in humanized mice (a model that expresses human ACE2 receptor), with clinical signs that are not reduced by SARS-CoV monoclonal antibody therapy or vaccination[7,8]. We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort)[34], suggesting active spillover. These data, phylogeographic analysis of SARSr-CoVs (Fig. 3), and coevoutionary analysis of bats and their CoVs (unpubl. data), suggest that bat caves in SW

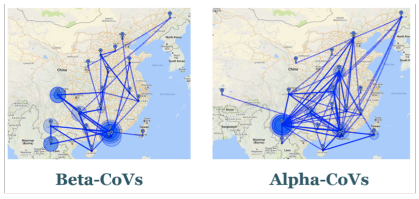

Figure 3. Ancestral location reconstruction for Beta- and Alpha-CoVs. The bigger the circle is, the more ancestral the corresponding node is.

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China, and *Rhinolophus* spp. bats are the likely origin of the SARS-CoV clade, and **therefore a clear-and-present danger for the re-emergence of SARS-CoV or a similar pathogenic virus.** The *Rhinolophus* spp. bats that harbor these viruses occur throughout SE Asia, across S. and W. Asia. **Thus, the geographic focus of DEFUSE is to use our research at this site to reduce the risk for the warfighter of these viruses spilling over across the region (West, South and SE Asia).**

Spatial models of bat origin high-risk viruses across S and SE Asia. We will build models that predict regional-scale bat and viral diversity in cave sites across South and SE Asia to enable warfighters and planners to estimate regional-scale risk from viral spillover based on locations. This will provide preliminary assessments for areas requiring greater on-the ground risk characterization to target deployment of viral suppression technologies. These regional-scale joint species distribution models (JSDM) will predict the composition of bat communities in caves in South Southern China, South and SE Asia. JSDMs use environmental and habitat data to predict the distributions of many species simultaneously, producing more accurate predictions than individual, separate species predictions by explicitly modeling positive and negative interactions between species and hidden factors such as shared habitat preferences. We will use a stochastic feedforward neural network to implement JSDMs that has proven effective at making predictions across multiple scales, with incomplete observations (as occurs for bats and their viruses), and explicitly accounting for bat species co-occurrence driven by shared environmental responses or evolutionary processes[35]. We will fit our JSDM to biological inventory data on over 200 caves in the region[36], using a combination of climatic and topographic variables including physiologically relevant bioclimatic variables (BIOCLIM) drawn from public, open source data sets[37], as well as proxies for subterranean habitat such as ruggedness and habitat heterogeneity. We will refine these models using regional-scale environmental variables (land-use, distance to roads, forest cover, degree of human disturbance etc.) and cave-specific variables (cave length, availability of roosting area, entrance dimensions, cave complexity, microclimate etc.). Our previous work has shown that these factors are predictors of bat species presence/absence at a given site[38]. Remote-sensing data and physical models will be used to estimate cave structures and microclimates where they are not available from biological inventory studies. We will validate our regional-scale species models using independent occurrence estimates and observations[39,40], including our extensive database on bat species occurrence in Southeast Asia [REF].

We will extend our predictions of bat communities to predictions of zoonotic disease risk using our unique species-level database of all known bat host-viral relationships[3] (Fig. 4); our >1800 viral detections from >20,000 individual bat samples in China and 7 other Asian countries (NIAID and USAID PREDICT); and results as they become available from a new 5-year DTRA-CBEP grant for field and lab investigations to characterize bat CoV diversity in Western Asia (Turkey, Jordan, Georgia, Pakistan, and Arabian Peninsula – EHA, Olival) to extend the

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geographic scope of our predictive models. We will use two strategies to predict presence of viruses at sites. Firstly, as a base case, we will assume that species have equal probability of carrying their known viral species across their range. Second, we will include viral species as additional outputs in our JSDM. We will fit this host-viral JSDM using data restricted to a smaller set of sites where both host species composition and viral detections are available. Based on performance of both models on hold-out data, we will determine which provides the best predictive power. For species composition and viral presence predictions, we will validate our models against a 20% validation subset of data that is held out for model validation, as well as data collected at our field sites in Task 3.

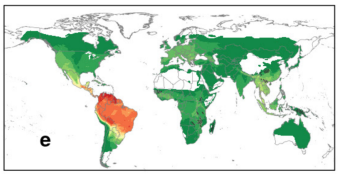

*Fig. 4: Predictive global map of total (known and unknown) viral diversity in bats (*Chiroptera *species). Based on EHA's unique database of all known mammal virus-host relationships[3].*

Commented [PD12]: This map doesn't help our case – superficial glance suggests we should be working in L. Am. I know this is incorrect, but I think we'd be better served putting in a map that highlights SW China as a hotspot… Could you just recreate this map only for Asia?. Can we also show a hotspot map of host distribution as well.

Prototype app for the warfighter. Drawing on experience building applications for data collection and analysis (e.g. https://flirt.eha.io/, https://eidr-connect.eha.io/, https://mantle.io/grrs), we will produce a prototype app for the warfighter that identifies the likelihood of dangerous viral pathogens spilling over from bats at a site. **The 'Viral spillover risk' app** will use outputs from our spatial risk modeling, data from EHA's extensive host-pathogen database, open-source species and pathogen ontologies, and app-directed crowd-sourced ultrasonic audio recordings to ground-truth and fine-tune its predictive capacity. This app will be updated in Y2 and Y3 to incorporate additional information on bat species-specific risk based on assays of host-virus binding and surveys of CoV prevalence. We will use risk-ranking algorithms developed by EHA (https://ibis.eha.io/) that use geolocation features, recency of information, and host and pathogen characteristics to display critical areas of high risk. The app will collect user GPS location data and preload bat species distribution and community composition estimates from our JSDMs. These will be refined with real-time surveillance data collected without the need to enter cave sites using field-deployable high-frequency microphones for bat detection[41]. We will combine reference acoustic calls from all bat species captured during proposed field work with existing data from bat call libraries globally to train species identification algorithms using bat echolocation call signatures. New algorithms using deep learning methods (e.g. convolutional neural networks[42]) will be developed, or adapted and externally validated on samples collected by the application to characterize bat species based on trained audio features. These models will be deployed on the mobile platform as they become available[42]. Bat species directly identified or estimated to occur within a scalable distance from the user will be automatically linked with viral diversity data from EHA's extensive host-pathogen database and with CoV sequence data from this project to deliver high-risk pathogen lists. The application will have 3 primary views; pathogens-

centric, bat-centric and map-centric. The pathogen-centric view will show a ranked list of likely pathogens in the user's current or selected location. The bat-centric view will show a ranked list of bat species for the user's location. The map-centric view will allow users to select a location for the other rank views, and will display a variety of map layers of interest, including heat map or distribution map layers profiling modeled or collected species occurrences around the user. Elements of the interface will be interactive, presenting popovers with more details when selected and displaying other map elements as appropriate. Alerts and notifications will give users a flexible way to monitor the app data passively, with the app proactively reaching out when critical information is received. The application will also offer a data collection module and accompanying interface elements to collect samples in the field and integrate collected data into the application database. The schemas, APIs, and protocols developed as part of this effort will be designed with principles of simplicity, interoperability, and usability in mind, including using RESTful URL schemes, and standardized data types and ontologies. Datasets will be hosted via cloud services from which the app will download updated information. Build and deployment processes will be reproducible, auditable, and transparent. All code modules will be continually available on EHA's GitHub page (LINK), be documented via README files in root directory of code repositories, and .zip archives containing code, datasets, and instructions for deployment will be made available. This will pave the way future incorporation of new structured biosurveillance data feeds and new species, viral, or host ontologies. This app will be designed for remote use (desktop platform) to assess specific sites in advance of personnel deployment on the ground, or in the field via mobile systems. This technology will improve overall situational awareness of existing and novel infectious agents found in bats, allowing DoD personnel to quickly identify areas that may pose the most significant risk for zoonotic spillover and rapidly deploy resources to respond to and mitigate their impact preemptively when necessary. The 'viral spillover risk' app will then be available to adapt for viral threats from other wildlife host species (e.g. rodents, primates) and ultimately for global use.

Full inventory of bat SARSr-CoV quasispecies at our cave test sites, Yunnan, China.
DEFUSE fieldwork will focus on three model cave test sites within a cave complex in Yunnan Province, SW China (MAP), where we have previously identified and isolated high-risk SARSr-CoVs able to infect human cells and cause SARS-like illness in mice[7,27,29,30]. At these sites, we will determine the baseline risk of SARSr-CoV spillover, prior to, during, and after our proof-of-concept field trials to reduce that risk. We will conduct longitudinal surveillance of bat populations to detect and isolate SARSr-CoVs, determine changes in viral prevalence over time, measure bat population demographics and movement patterns, to definitively characterize their SARSr-CoV host-viral dynamics. We will sample *Rhinolophus, Hipposideros*, and *Myotis* species, all of which carry SARSr-CoVs, and co-roost in the same caves[3,36]. Surveillance will be conducted before, during, and after deployment of our intervention field trial (Task X) to

establish baseline viral shedding detection rates and measure the impact of treatment on these. Field data will allow us to test the accuracy of our model predictions and compare the efficacy of laboratory trials in animal models with in-the-field trials.

Our test caves near Kunming, Yunnan Province, contain multiple co-roosting *Rhinolophus*, *Hipposideros*, and *Myotis* spp., although our preliminary data demonstrate that *R. sinicus* and *R. ferrumequinum* (which co-roost at our sites) are the SARSr-CoV primary reservoir, with *Hipposideros* and *Myotis* playing an insignificant role in viral dynamics. We will capture bats using harp traps and mist nets during evening flyout. Rectal, oral, and whole blood samples (**×2** per bat) will be collected for viral discovery using sterile technique to avoid cross-contamination. 2-mm wing tissue punch biopsies will be collected from each bat for host DNA bar-coding, sequencing of host ACE-2 receptor genes (interface site), and cophylogeny analyses. Standard morphological and physiological data will be collected for each bat (age class, sex, body weight, reproductive status etc.). In Phase I we will **sample 60** *Rhinolophus sinicus* and **60** *R. ferrumequinum*, our primary target species, (**120** bats total) **every three months** for non-lethal viral specimen collection over an **18 month period** of the project from all three cave sites. Given the average prevalence of SARSr-CoV in these species in our previous investigations in S. China (~6-9%, n=3304 *Rhinolophus* spp.), this sample size would enable to detect changes of 10% fluctuation in prevalence between sampling periods. Early in the sampling we will trial the efficacy of tarp collection of fresh feces and urine as a way of collecting viral dynamics data while reducing roost disturbance (REFS). To identify seasonal or reproductive cycle variation in viral dynamics, we will conduct repeated sampling of individuals and of tarps placed under the same roost site portion of a cave and examine roost-site fidelity (see below) to measure how well tarp-collected samples will track the general population. *Rhinolophus* species have a 7-week gestation period and generally give birth in the spring. Colony composition may change over the year, with bats aggregating during mating periods. These changes will affect viral dynamics and our sampling strategy will allow us to collect data over two mating and gestation periods and assess changes in viral prevalence. Additionally, we will conduct **pre-intervention (3 months prior to deployment) and post-intervention (3 months following deployment) CoV monitoring from these sites in Phase II (see Fig. X -Gantt chart)** to assess efficacy of our field intervention deployment. During months without physical bat trapping (2 months each quarter of sampling), fresh fecal pellets will be collected by placing clean polyethylene sheets measuring 2.0m x 2.0m beneath roosting bats. We will use infrared spotlights and digital infrared imaging to record the number and species of individuals above each plastic sheet. Fecal pellets may also be genetically barcoded to confirm species identification[43] as we routinely do for other bat surveillance projects. All specimens will be preserved in viral transport medium and immediately frozen in liquid nitrogen dry shippers in the field, then transported to partner laboratories with maintained cold chain and strict adherence to biosafety protocols. Each bat will be marked with a subcutaneous microchip (PIT tag) containing

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Commented [15]: 3 cave sites will be the same across the entire project, one cave will later be experimental cave for intervention with 2 control caves. If there aren't enough bats in any given cave, we can add additional cave sites to get our target sample sizes, e.g. 2 adjacent caves sampled instead of one to get 120 bats per event.

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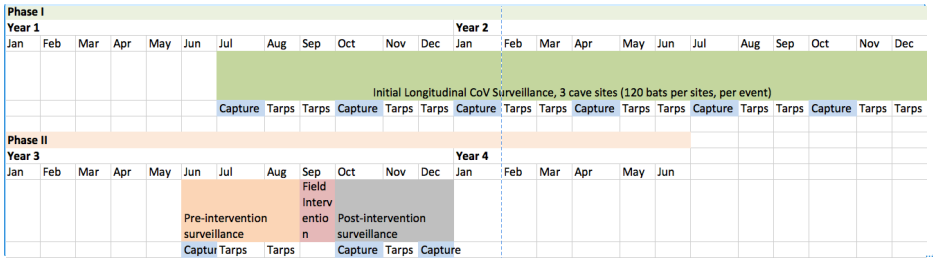
a unique ID number (**see below**). **Study caves and bat roosts will be surveyed using portable LiDAR technology**[44-46], to give a 3-D image of the roost area which will provide data on species composition and volume/surface area that needs to be covered when applying the immune treatments in TA2 (Fig. XX). We will adjust individual sampling quotas per species to optimize viral detection based on host-specific prevalence of previous and ongoing host-pathogen models, as well as ongoing lab results from bat sampling.

Our team has more than 30 years of collective experience in safe and humane handling of bats for biological sampling. This project will operate under appropriate IACUC/ACURO and PPE guidelines. EHA has several ongoing DTRA-supported projects and is familiar with the process of obtaining ACURO approval for animal research from the DoD. The EHA team also currently maintains IACUC protocols through Tufts University (via inter-institutional agreement) and will obtain IACUC approval through this mechanism for DEFUSE.



Bats are highly mobile and little is known of inter-cave migration/emigration rates. To **monitor bat roost fidelity and movement** we will mark *Rhinolophid* bats with individual Passive Integrated Transponder (PIT) tags to track individual bats' entry and exit from roost caves. Tags will be inserted subcutaneously between the bats' scapulae by trained personnel. The identities of individually tagged bats inhabiting roost caves will be recorded using radio frequency identification (RFID) data loggers and antennae at the roost entrances. Time-stamped data from individual bats collected by data loggers will be downloaded every 3 days to examine temporal roost site fidelity and rates of inter-cave immigration/emigration. Infrared video cameras will record the total number of bats flying out each night. Recapture data will be collected continuously throughout the project. We will attach radio transmitters (1.2g, Advanced Telemetry Systems, MN USA), to the back of 20 individual *Rhinolophus sinicus* and *Rhinolophus ferrumequinum* from each study roost (60 total) to determine nightly foraging patterns and local dispersal patterns. Telemetry data and PIT tag data will be used to calculate home range, to determine the degree of mixing among our three sites, and parameterize our dynamic models. We will use fine scale data on roost fidelity to determine the population mix at the specific roost sites (e.g. a side pocket of a cave where only one species roosts) for our

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intervention. Radio transmitters that weigh <3% of bat body weight will be attached to the fur on the back using a veterinary dermatological adhesive (Vet Bond 3M, USA). We will collect location data from 60 bats (30 males, 30 females) every day for 10 days, 3 times per year for the 18 months of Phase 1. This will provide seasonal data to assess movement, including mating and gestation periods when higher levels of mixing and aggregation in the caves are expected.

High-risk SARSr-CoV quasispecies discovery, isolation and S. gene characterization. We will screen samples for SARSr-CoV nucleic acid using our pan-coronavirus consensus one-step hemi-nested RT-PCR (Invitrogen) assay targeting a 440-nt fragment in the RNA-dependent RNA polymerase gene (RdRp) of all known alpha- and betacoronaviruses assay[47,48], as well as specific assays for known SARSr-CoVs[27-30]. PCR products will be gel purified and sequenced with an ABI Prism 3730 DNA analyzer and quantitative PCR will be performed on SARSr-CoV-positive samples to determine viral load. Full-length genome of all detected SARSr-CoVs will be sequenced by high throughput sequencing method followed by genome walking. The sequencing libraries are constructed using NEBNext Ultra II DNA Library Prep Kit for Illumina and sequenced on a MiSeq sequencer, with PCR and Sanger sequencing used to fill gaps in the genome[29,30,32]. We will build phylogenetic trees using the Maximum Likelihood algorithm in the PhyML software, then scan for recombination events using Recombination Detection Program (RDP), confirmed using similarity plot and bootscan analyses in Simplot. We will analyze the S gene (which encodes the spike protein and determines receptor binding and cross-species transmission) of each sequence to identify a virus' potential to use human molecule ACE2 as a receptor. SARSr-CoVs with high similarity with SARS-CoV in full-length genomic sequences or with S proteins likely able to use human ACE2 as receptor will be identified as potential high-risk strains. We will then attempt isolation, cell culture, and infectious clone construction for further study *in vivo* and *in vitro* analysis. We have had success isolating and culturing SARSr-CoVs using Vero E6 monolayers in DMEM medium with 10% FCS, confirmed by RT-PCR and electron microscopy[29]. For SARSr-CoVs which we are not able to culture, we will construct recombinant viruses with the S gene of new bat SARSr-CoVs and the backbone of the infectious clone of SARSr-CoV WIV1 or of SARS-CoV, using the reverse genetic system described previously, and detailed below[28]. Initial assays of receptor usage and cell tropism will use various cell lines expressing human ACE2 incubated with isolated bat SARSr-CoVs or pseudotype viruses as previously shown[29].

Approach to predicting bat SARSr-CoV spillover risk. Our approach is to combine state-of-the-art genotype-phenotype modeling with detailed step-wise experimental characterization of each bat SARSr-CoV we identify at our test cave sites.
Flow chart here:
Sample testing/screening/Isolation – phylogenetic analysis/ACE2 binding modeling – ACE2

binding assays (all from Fig A) – chimera production – mouse model – SARS vaccines protect - cross neut humAB – full length recovery (all from Fig b)-) – Data into predictive modeling (additional box)

This flow chart should use some elements of Ralph's figures A and B as indicated. Ask Ralph to send you Figs A and B in editable format so you can fuse them in the way above (a chimera!), and without the text. The flow chart needs to have less detail so the flow is visible when shrunk down.

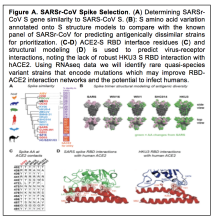

Figure A. SARSr-CoV Spike Selection. (**A**) Determining SARSr-CoV S gene similarity to SARS-CoV S. (**B**): S amino acid variation annotated onto S structure models to compare with the known panel of SARSr-CoV for predicting antigenically dissimilar strains for prioritization. (**C-D**) ACE2-S RBD interface residues (**C**) and structural modeling (**D**) is used to predict virus-receptor interactions, noting the lack of robust HKU3 S RBD interaction with hACE2. Using RNAseq data we will identify rare quasi-species variant strains that encode mutations which may improve RBD-ACE2 interaction networks and the potential to infect humans.

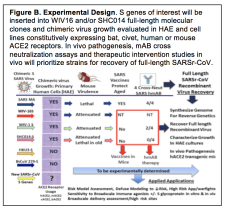

Figure B. Experimental Design. S genes of interest will be inserted into WIV16 and/or SHC014 full-length molecular clones and chimeric virus growth evaluated in HAE and cell lines constitutively expressing bat, civet, human or mouse ACE2 receptors. In vivo pathogenesis, mAB cross neutralization assays and therapeutic intervention studies in vivo will prioritize strains for recovery of full-length SARSr-CoV.

Our models will be parameterized with the experimental data from a series of assays on the S genes of bat SARSr-CoVs, with experimental and modeling work flowing together in iterative steps. The Baric laboratory pioneered many of the experimental approaches, the SARSr-CoV reverse genetic platforms, and full length S chimeric recombinant virus recovery from in silico sequence databases[7,8,23,49]. Full length recombinant strains reconstructed using reverse genetics in our lab include human epidemic strains, civet and raccoon dog SARS-CoV strains, and bat SARSr-CoVs (WIV16, WIV1, SHC014 and HKU3-SRBD repaired RBD interface). These strains will be used in the Baric, Shi and Wang laboratories for initial work on immune boosting and priming, and act as baseline data to parameterize the spillover risk modeling[7,8,23,49]. They will be supplemented by viruses we isolate under DEFUSE (worked on in the Shi lab) **and approximately 15-20 bat SARSr-CoV spike proteins/year** from DEFUSE (Baric, Shi labs). Most of the ~150 bat SARSr-CoV strains sequenced by us in prior work have not yet been examined for spillover potential and these will also be assessed in the following pipeline:

Experimental assays of SARSr-CoV spillover potential: *Ability to enter human cells:* Viral entry represents the key first step to evaluating the disease potential of SARSr-CoVs, with CoV species-specific restriction occurring primarily at entry[23,49]. To assess this we first will use structural modeling of SARSr-CoV S protein to ACE2 receptors. The structure of the SARS trimer prefusion S and the bound SARS-CoV S RBD to human and civet ACE2 have been solved, providing a platform for structural modeling and mapping hot spots of antigenic variation[50,51]. Mutations in the RBD[23,49,52,53], and host proteases and S glycoprotein proteolytic processing[54-56], regulate SARSr-CoV cell entry and cross-species infectivity. Mismatches in the S-RBD-ACE2 molecules or S proteolytic processing will prevent cell entry of SARS-CoV[23,49]. We will also

conduct *in vitro* pseudovirus binding assays, as we have done previously for WIV1 and others[29], as well as live virus binding assays for strains we are able to isolate. This work will be done in China (Shi lab), to prevent delays and unnecessary dissemination of viral cultures.
Novel SARSr-CoV Virus Recovery: We will commercially synthesize select SARSr-CoV S glycoprotein genes, designed for insertion into our SHC014 or WIV16 molecular clone backbones (these viruses are 88% and 97% identical to epidemic SARS-Urbani in the S glycoprotein). These are BSL-3, not select agents, and pathogenic in hACE2 transgenic mice. Different backbone strains provide increased opportunities for recovery of viable viruses, and to identify potential barriers for RNA recombination-mediated gene transfer between strains[30]. Chimeric viruses will be recovered in Vero cells, or in mouse cells over-expressing human, bat or civet ACE2 receptors to support cultivation of viruses with a weaker RBD-human ACE2 interface. All chimeric viruses will be sequence verified and evaluated for: **i**) human, civet and bat ACE2 receptor usage *in vitro*, **ii**) growth in primary HAE, **iii**) sensitivity to broadly cross neutralizing human monoclonal antibodies (mAB) S215.17, S109.8, S227.14 and S230.15 and a mouse antibody (435) that recognize unique epitopes in the RBD[57,58] and **iv**) *in vivo* pathogenesis studies in hACE2 transgenic mice, using our well established approaches[7]. Should some isolates prove highly resistant to our mAB panel, we will evaluate cross neutralization against a limited number of human SARS-CoV serum samples from the Toronto outbreak in 2003 (n=10). Chimeric viruses that encode novel S genes with spillover potential (e.g. growth in HAE, use of multiple species ACE2 receptor for entry, antigenic variation) will be used to identify SARSr-CoV strains for recovery as full genome length viable viruses. *Recovery of Full length SARSr-CoV:* We will compile sequence/RNAseq data from a panel of closely related strains (e.g.<5% nucleotide variation) and compare the full length genomes, scanning for unique SNPs representing sequencing errors[59-61]. The genome of consensus candidates will be synthesized commercially (e.g. BioBasic), as six contiguous cDNA pieces linked by unique restriction endonuclease sites for full length genome assembly. Full length genomes will be transcribed into genome-length RNA and electroporation used to recover recombinant viruses[22,62]. We will re-evaluate virus growth in primary HAE cultures at low and high multiplicity of infections and *in vivo* in hACE2 transgenic mice, testing whether backbone genome sequence alters full length SARSr-CoV spillover potential. All experiments will be performed in triplicate and data provided to the Modeling Team in real time. We anticipate recovering ~3-5 full length genomes/yr, reflecting strain differences in antigenicity, receptor usage, growth in human cells and pathogenesis. *In vivo Pathogenesis:* We generated a mouse that expresses human ACE2 receptor under control of HFH4, a lung ciliated epithelial cell promoter[7]. Infection of this model with wildtype SARS-CoV results in lethal disease, but transient disease with bat SARSr-CoV WIV1, suggesting that WIV1 is less efficient at using hACE2 *in vivo* and less likely to produce severe disease in people initially on spillover. However, single amino acid variations in the SARS-CoV RBD of related strains could dramatically alter

these phenotypes, hence we will evaluate the impact of low abundant, high consequence micro-variation in the RBD. Groups of 10 animals will be infected intranasally with 1.0 x 10^4 PFU of each vSARSr-CoV, then clinical disease (weight loss, respiratory function by whole body plethysmography, mortality, etc.) followed for 6 days p.i.. Animals will be sacrificed at day 2 or 6 p.i. for virologic analysis, histopathology and immunohistochemistry of the lung and for 22-parameter complete blood count (CBC) and bronchiolar alveolar lavage (BAL) using the Vetscan HM5 (an instrument that measures parameters used for human clinical determination). *Identification of high risk/low abundant variants:* We will use RNAseq to identify low abundant quasispecies (QS) variants encoding mutations in RBD and/or residues that bind ACE2. These would alter risk assessment calculations as strains identified as low risk, might actually have low abundant, high risk variants circulating in the QS. To test this the Shi and Baric lab will structurally model and identify highly variable residue changes in the SARSr-CoV S RBD and use commercial gene blocks to introduce these changes singly and then in combination into the S glycoprotein gene of the low risk, highly abundant parental strain. We will examine the capacity of these low abundance chimeric viruses to use human, bat, civet and mouse ACE2 receptors, and to replicate in HAE cultures. *RBD deletions:* Small deletions at specific sites in the SARSr-CoV RBD leave the key RBD-ACE2 interface residues intact, such that Clade 1 strains represent higher risk of human infection (Fig. 5). We will analyze the functional consequences of these RBD deletions on SARSr-CoV hACE2 receptor usage, growth in HAE cultures and *in vivo* pathogenesis. First, we will delete these regions, sequentially and then in combination, in SHC014 and SARS-CoV Urbani, anticipating that the introduction of both deletions will prevent virus growth in Vero cells and HAE. We hypothesize that the smaller deletion may be tolerated, given its location in the RBD structure, so *in vivo* passage in the presence of receptor will restore growth, while identifying 2nd site reversions that restore efficient hACE2 usage[49]. In parallel, we will evaluate whether RBD deletion repair restores the ability of low risk strains to use human ACE2 and grow in human cells. To test this we will synthesize full length rs4237, a highly variable SARSr-CoV that encodes a few of the SHC014 RBD contact interface residues but also encodes a mutation at 479 (N479S) and has two deletions and hence, is not recoverable *in vitro*. Using the SHC014 backbone sequence, we will sequentially and then in tandem repair the deletions in the presence and absence of the S479N. We anticipate that the S479N mutation is critical given its key role in establishing the RBD-ACE2 interface, and that restoration of the RBD deletions will enhance virus recognition of hACE2

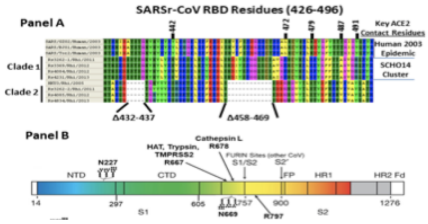

Figure C. Additional Markers for High/Low Risk Strain Identification. (**A**). Clade 2, but not Clade 1 SARSr-CoV encode programmed deletions which likely impact ACE2 receptor usage. (B) Proteolytic and N-glycosylation sites of interest.

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receptors and growth in Vero cells and HAE cultures *S2 Proteolytic Cleave and Glycosylation Sites:* After receptor binding, a variety of cell surface or endosomal proteases[63-66] cleave the SARS-CoV S glycoprotein causing massive changes in S structure [67] and activating fusion-mediated entry[55], which is prevented in the absence of S cleavage[68] (Fig. 5). Tissue culture adaptations sometimes introduce a furin cleavage site which can direct entry processes, usually by cleaving S at positions 757 and 900 in S2 of other CoV, but not SARS[66]. For SARS-CoV, a variety of key cleavage sites in S have also been identified and we will analyze all SARSr-CoV S gene sequences for appropriately conserved proteolytic cleavage sites in S2 and for the presence of potential furin cleavage sites[69,70]. SARSr-CoV S with mismatches in proteolytic cleavage sites can be activated by exogenous trypsin or cathepsin L. Where clear mismatches occur, we will introduce the appropriate human-specific cleavage sites and evaluate growth potential in Vero cells and HAE cultures. In SARS-CoV, we will ablate several of these sites based on pseudotyped particle studies and evaluate the impact of select SARSr-CoV S changes on virus replication and pathogenesis (e.g. R667, R678, R797). We will also review deep sequence data for low abundant high risk SARSr-CoV that encode functional proteolytic cleavage sites, and if so, introduce these changes into the appropriate high abundant, low risk parental strain. *N-linked glycosylation:* SARS-CoV S has 23 potential N-linked glycosylation sites and 13 of these have been confirmed biochemically. Several of these regulate SARS-CoV particle binding DC-SIGN/L-SIGN, alternative entry receptors for SARS-CoV entry into macrophages/monocytes[71,72]. Mutations that introduced two new N-linked glycosylation sites may have been involved in the emergence of human SARS-CoV from civet and raccoon dogs[72]. While the sites are absent from civet and raccoon dog strains as well as clade 2 SARSr-CoV, they are present in WIV1, WIV16 and SHC014, supporting a potential role for these sites in host jumping. To evaluate this, we will sequentially introduce clade 2 residues at positions N227 and N699 of SARS-CoV and SHC014 and evaluate virus growth in Vero cells, nonpermissive cells ectopically expressing DC-SIGN and in HAE cultures, as well as in human monocytes and macrophages anticipating reduced virus growth efficiency. Using the clade 2 rs4237 molecular clone, we will introduce the clade I mutations that result in N-linked glycosylation sites at positions 227 and N699 and in rs4237 RBD deletion repaired strains, evaluating virus growth efficiency in HAE, Vero cells, or nonpermissive cells ± ectopic DC-SIGN expression[72]. *In vivo*, we will evaluate pathogenesis in transgenic ACE2 mice.

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Models to predict viral spillover potential and evolution of high-risk SARSr-CoV strains. *Structural equation model of spillover potential:* We will use data from the experimental assays above to **build genotype-phenotype models of bat SARSr-CoV spillover potential**. We will use Bayesian Structural Equation Models (SEM), fit via MCMC methods[73], to predict spillover potential from the genetic traits of bat SARSr-CoVs and the ecological traits of hosts. SEMs have successfully analyzed the drivers of, and predicted stochastic species interactions[74,75]. They will

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enable us to integrate multiple, interrelated tests of strain spillover potential into a common framework, while restricting relationships to plausible causal pathways. This prevents the over-fitting associated with a black-box approach. A Bayesian approach allows fitting with unbalanced and non-independent data, as per the larger number of cell-binding and cell-entry assays we will run to determine candidates for a smaller number of humanized mouse trials and LIPS assays (below). The viral traits derived from the experimental assays of spillover risk laid out above will be our primary set of predictor variables: presence of deletions in the RBD region, proteolytic binding sites, glycosylation sites, neutralization escape mutations, indeterminate mutations at high-variation sites found in low-abundance strains. We will include genetic similarity of each strain's RBD to the reference pandemic SARS-CoV genomes to test these aggregate measures as predictive proxies. To control for experimental conditions we will include whether assays were performed on live viral isolates, full-genome or synthetic chimeric viruses, and the molecular backbone used in the latter. These traits will be used as inputs to SEM's causal graph, and used to predict latent variables representing the interconnected processes that contribute to SARSr-CoV QS spillover potential: receptor binding, cell entry with and without the presence of exogenous proteases, immune system interaction, and intracellular growth, all measured by our laboratory assay. These, in turn will **act as predictors for the ultimate outcomes of host pathogenesis (Fig. 6)**. We will use previous work on these genetic traits to put informative priors on strength and direction of interactions in the causal graph. We will use **prior-knowledge model simulations to select target sequences from our sampling for characterization and genome-sequencing**, to collect data that maximally enhances the predictive power of our model. We will use regularizing priors to reduce over-fitting and help select the most predictive variables in the final predictive model.

Evolutionary modeling and simulation to predict potential strains: Our SEM modeling will generate estimates of the spillover potential of SARSr-CoV sequences from DEFUSE fieldwork and prior work. To examine risk associated with the total viral population at our test sites, we will model and simulate evolutionary processes **to identify likely viral QS that our sampling has not captured, as well as viral QS likely to arise in the future**. By estimating the spillover potential of these simulated QS, we can better characterize the risk associated with the total viral population. We will use a large dataset of S protein sequences and full-length genomes generated from prior work and DEFUSE fieldwork to estimate SARSr-CoV substitution rate and its genome-wide variation using coalescent and molecular clock models within a Bayesian MCMC framework[76]. We will then estimate SARSr-CoV recombination rates at the cave population level using the same dataset and Bayesian inference[77,78]. We will apply various methods (RDP[79], similarity plots, bootscan) to identify recombination breakpoints and hotspots within the SARSr-CoV genome. Using these estimates of substitution and recombination rates, we will simulate the evolution of the SARSr-CoV QS virome using a forward-time approach implemented in simulators that model specific RNA virus functions (e.g. VIRAPOPS[80]). This will

allow us to predict the rate at which new combinations of genetic traits can spread in viral populations and compare recombination rates among caves and bat communities. Our forward-simulated results **will provide a pool of likely unknown and future QS species**. Using these and our SEM model for spillover risk, **we will predict the QS that are most likely to arise *and* have pathogenetic and spillover potential.** We will use the evolutionary simulation results to iteratively improve our SEM model results. The number of genetic traits of interest for prediction of pathogenicity is potentially large, so we will perform variable reduction using tree-based clustering, treating highly co-occurring traits as joint clusters for purposes of prediction. We will generate these clusters from our full set of SARSr-COV sequences from DEFUSE fieldwork and prior work. However, as trait clusters may be modified in future virus evolution due to recombination, we will use our forward-evolutionary modeling to predict how well trait clusters will be conserved, retaining only those trait clusters unlikely to arise in unknown or future viral QS genomes. This will enable a good trade-off between increased predictive power based on current samples and generalizability to future strains that have not yet evolved.

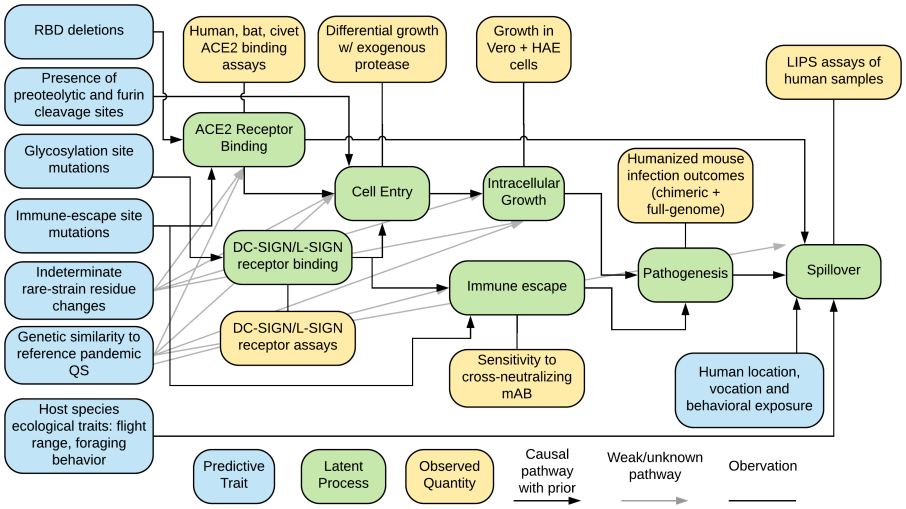

Figure 6: A simplified directed graph of a structural equation model representing the causal relationships between predictors and measures of viral pandemic potential.

Validation by LIPS assay on previously-collected human sera: Following our proof-of-concept field trial we will update these models to include not only pathogenesis but spillover probability validated with data on viral QS antibodies found in the local human population detected via Luciferase immunoprecipitation system (LIPS) assays on previously-collected human sera (NIAID project, Daszak PI). This includes >2,000 samples collected from people living close to our test

cave sites in Yunnan Province, and is the basis of a recent paper demonstrating 2.7% seropositivity to bat SARSr-CoVs in an initial sampling of this population[34] (Fig. 7). In addition to serum samples, extensive behavioral and wildlife contact data has been collected from this population, under an IRB that can be easily extended to cover DEFUSE work.

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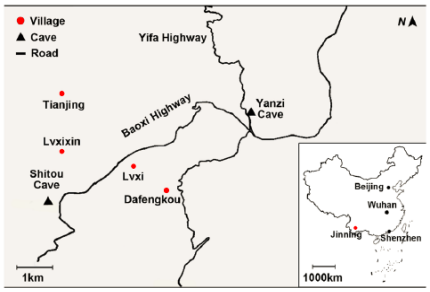

Fig. 7. Human sera were collection from villages (red dots) near bat caves where CoV positive samples have been isolated (Yanzi Cave and Shitou Cave, triangle).

Our ability to extend and validate these models with data on actual human contact and spillover allows us to fit and test models of actual, not just potential, spillover probability. Our previous work has shown that both host and viral traits predict zoonotic spillover from models[3], so in addition to viral traits, we will include key ecological traits of the host bat species in which viral QS were detected. These include flight ranges, foraging, roosting, demographic, and social behavior. To will use the extensive data on each person's behavioral exposure to wildlife, and their work, travel and occupational history, to correct for varying human exposure to bat species. We will design LIPS assays for specific high- and low-spillover risk SARSr-CoVs, to identify people who've been exposed to them, and test our model's validity. The LIPS uses viral antigens tagged with luciferase, from crude lysate, thereby eliminating the requirement for antigen purification and significantly reducing the time required for assay development and producing a more sensitive test than traditional ELISA[81]. Prof. Zhengli Shi (Wuhan Institute of Virology) will lead the LIPS serological work based on her 15 years SARSr-CoV human serological surveillance experience [82-84] and the recent success in SADS-CoV zoonotic risk study using LIPS[85]. To establish SARSr-CoV LIPS assays, we will: **1)** Insert different high- and low-risk SARSr-CoV N genes into pREN-2 vector (LIPS vector). We will first assess N gene similarity to determination their potential cross-reactivity in a LIPS assay. From our previous experience, SARSr-CoV maintain 80% similarity in the N protein, thus should be detectable using a universal SARSr-CoV N based LIPS assay; **2)** determine specificity of the LIPS assay by producing polyclonal sera via injection of recombinant protein or attenuated virus into rabbits. Selected SARSr-CoV N proteins or viral particles will be used as the immunogen for antibody production; **3)** validate SARS-CoV, MERS-CoV and SADS-CoV N protein LIPS assays by incubating antigens with their respective positive serum samples and the antigen antibody complex eluted using protein A/G beads. Luminescence is measured upon adding coelentrazine, a substrate of renilla luciferase. In a preliminary assay, LIPS successfully detected high strong antibody titer in the positive control serum sample, while the vector control did not show any response. Cut off was set as the average luminescence plus

three standard deviation from the control. We have used this to demonstrate efficacy for MERS-CoV and SADS-CoV (Fig. 8); **4)** validate LIPS positive sera results by spike protein based LIPS and viral neutralization assay. Similarly, S gene from high/low risk SARSr-CoV will be engineered into the pREN-2 vector and an S-LIPS assay produced, as above. As a confirmatory test the positive samples from LIPS, will be validated by viral neutralization assay. The data from LIPS and neutralization will be collected and analysis to validate the model.

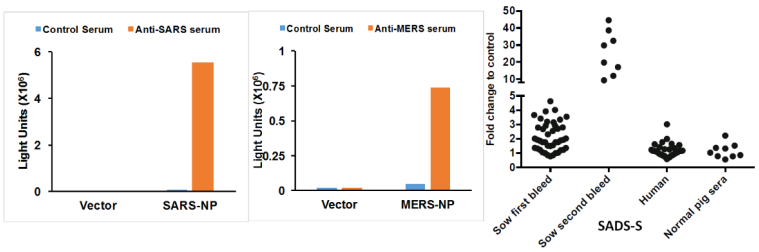

Fig. 8. LIPS assay was tested successful for SARS, MERS and SADS coronavirus N or S antibodies.

Thematic Area 2

Immune modulation approach to reducing bat SARSr-CoV spillover risk. There is no available technology to reduce the risk of exposure to novel CoVs from bats which carry zoonotic precursors to many emerging viruses including filoviruses (Ebola), CoV (SARS-CoV, MERS-CoV, etc.), paramyxoviruses (Nipah/Hendra), rhabdoviruses (rabies) and others. No vaccines or therapeutics exist for emerging CoVs, filoviruses and paramyxoviruses and exposure mitigation strategies are non-existent. We have shown that bats have unique immunological features that may explain why they coexist with viruses and rarely show clinical signs of infection. Our long-term studies demonstrate: a) bats maintain constitutively high expression of IFNα that may respond to and thus restrict, viral infection immediately[11]; b) several bat interferon activation pathways are dampened, e.g. STING (a central cytosolic DNA-sensor molecule to induce interferon) dependent and TLR7 dependent pathways[10]; c) the NLRP3 dependent inflammasome pathway is dampened, and some of the key inflammation response genes like AIM2 have been lost in bats[86,87]. The dampened IFN and inflammasome response suggest bats maintain a fine balance between IFN response and detrimental over-response. This is likely due to an adaptation of their immune-sensing pathways as a fitness cost of flight[9]. We hypothesize that the bat innate/adaptive immune responses are quite different from that of human and mouse. Firstly, virus replication will likely be restricted quickly by constitutively expressed IFNα in bats, resulting in lower B/T cell stimulation due to lower viral stimuli. Second, dampened interferon and inflammasome responses will result in lower cytokine responses that are

required to trigger T/B cell dependent adaptive immunity (e.g. antibody response). The strong innate immune response, due to the lack of an efficient antibody response, will clear the virus. **We and others have demonstrated proof-of-concept of this phenomenon:** Experimental Marburg virus infection of Egyptian fruits bats, a natural reservoir host, resulted in wide tissue distribution yet low to moderate viral loads, brief viremia, low seroconversion and a low antibody titer that waned quickly, suggesting no long-term protection is established[88-90]. Similarly, poor neutralizing antibody responses occur after experimental infection of bats with Tacaribe virus[91] and in our studies with SARS-CoV experimentally infected bats (L-F Wang, unpublished data). Indeed, we successfully showed bat interferon can inhibit bat SARSr-CoVs[28]. We hypothesize that if we can use immune modulators that upregulate the naturally low innate immunity of bats to their viruses, we will be able to transiently suppress viral replication and shedding, reducing the risk of spillover. We will evaluate two immune modulation approaches to defuse spillover of SARSr-CoVs from bats to humans: **1) Broadscale Immune Boosting strategies (Wang, Duke-NUS):** we will apply immune modulators like TLR-ligands, small molecule Rig like receptor (RLR) agonists or bat interferon in live bats, to up-regulate their innate immunity and assess suppression of viral replication and shedding; **2) Targeted Immune Priming (Baric, UNC):** the broadscale immune boosting approach will be applied in the presence and absence of chimeric immunogens to boost clearance of high-risk SARSr-CoVs. Building on preliminary development of polyvalent chimeric recombinant SARSr-CoV spike proteins, we will use novel chimeric polyvalent recombinant S proteins in microparticle encapsidated gels and powders for oral delivery and/or virus adjuvanted immune boosting strategies where chimeric recombinant SARSr-CoV S are expressed by raccoon poxvirus, which has been used extensively to deliver rabies immunogens in bats and other animals. We will conduct application trials with live bats to assess suppression of replication and shedding of a broad range of pathogenic SARS-related CoVs. Both lines of work will begin in Year 1 and run parallel, be assessed competitively for efficiency, cost, and scalability, and successful candidates used in our live bat trials at our test sites in Yunnan, China. We believe an immune boosting/priming strategy is a superior approach for this challenge because solutions are likely to be broadly applicable to many bat species, and across many viral families.

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Broadscale immune boosting (led by Wang, Duke-NUS). We will work on the following key leads to identify the most effective approach to up-regulate innate immunity an suppress viral loads. *Toll-like receptor (TLR)/Rig-I Like Receptor (RLR) ligands:* We have begun profiling bat innate immune activation *in vivo*, in response to various stimuli. Our work indicates a robust response to TLR-stimuli like polyI:C when delivered *in vivo*, as measured by transcriptomics on spleen tissue (Fig. 7). We have performed transcriptomics on spleen, liver, lung and lymph node, with matched proteomics to characterize immune activation *in vivo*. These activation profiles will be used to assess the bat immune response to different stimuli and direct the

response to favor those which lower the viral load in our experimental system at Duke-NUS (below). In addition to the ligands already tested, we will stimulate the Rig-I pathway with 5'pppDSRNA, a mimetic of the natural RIG-I stimulant. These stimulants will activate functional bat IFN production pathways, and a similar strategy has been demonstrated in a mouse model for clearance of SARS-CoV, influenza A virus and Hepatitis B virus[12,15].

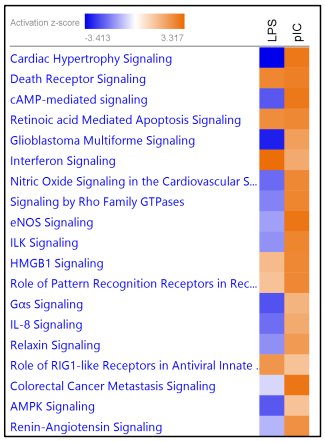

Fig. 7. Pathway analyses from Ingenuity Pathway Analysis (IPA) of whole spleen NGS after stimulation with either LPS or polyI:C. Z-score increase over control bats is indicated as per scale, and suggests strong activation of many pathways.

Universal bat interferon: To overcome any complications arising from species-specificity, we will design a conserved universal bat interferon protein sequence and produce purified protein. Utilization of a universal IFN for bats will overcome species-dependent response to the ligand, allowing the use of IFN throughout broad geographical and ecological environments and across many bat species. As a starting point, we have produced recombinant non-universal, tagged, bat IFN that are effective at inducing appropriate immune activation (Fig. 8). This ligand can be **delivered by aerosol or intranasal application** as has been shown to reduce viral titers in humans, ferrets and mouse models[12,13,15]. Interferon has been used clinically in humans as an effective countermeasure when antiviral drugs are unavailable, e.g. against filoviruses[14]. Replication of SARSr-CoV is sensitive to IFN treatments, as shown in our previous work[28]. The successful delivery, immune activation and outcome on the host will be characterized thoroughly to optimize rapid immune activation.

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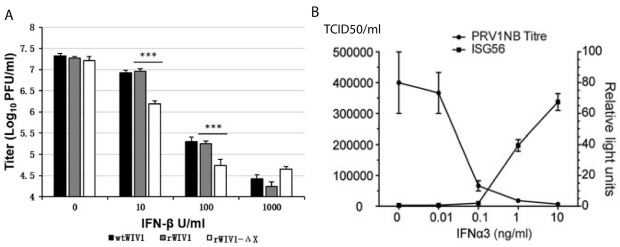

Fig. 8: Bat viruses are sensitive to IFN treatments. A) Recombinant bat SARS-related coronavirus WIV1 replication was inhibited by human IFN-β in a dose dependent manner in Vero cells. B) Bat reovirus PRV1NB replication was inhibited by recombinant bat IFNα3 in a dose dependent manner in bat PakiT03 cells.

Boosting bat IFN by blocking bat-specific IFN negative regulators: Uniquely, bat IFNα is naturally constitutively expressed but cannot be induced to a high level, indicating a negative regulatory factor in the bat interferon production pathway[92]. To fast-track the identification of this target

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we will utilize a *Pteropus alecto* CRISPRi library pool that we have created covering multiple RNA targets in every gene in the *P. alecto* genome. The library has already been produced and genes affecting influenza replication in bat cells have been identified. Using CRISPRi we can identify negative regulator genes and then screen for compounds targeting these genes to boost the inducibility of the IFN system in a shorter time-frame. Based on previous work, it is highly likely this will be a conserved pathway throughout the order *Chiroptera*. *Activating dampened bat-specific innate immune pathways which include DNA-STING-dependent and TLR-dependent pathways:* Our work showing that mutant bat STING or reconstitution of AIM2 and functional NLRP3 homologs restores antiviral functionality suggests these pathways are important in bat-viral coexistence and that the majority of the pathway is preserved. By identifying small molecules to directly activate pathways downstream of STING or TLR/RLRs, such as TBK1 activation, we will activate bat innate defense by interferons and promote viral clearance. We hypothesize that these small molecules we will be able to significantly reduce viral load in bats. *Validation in a bat-mouse model.* Various CoVs show efficient infection and replication inside the human host but exhibit defective entry and replication using mouse as a host due in part to differences in DPP3 and ACE2 receptors. We have shown efficient reconstitution of irradiated mice using bat bone marrow from multiple species, including *E. spelaea*. Fig. 9 shows the efficient reconstitution of bat PBMC's in the mouse, presence of circulating bat cells and generation of bat-specific antibodies in mice incapable of producing an antibody response. This 'batized' mouse model can be utilized for both circulating infection of SARS/MERS CoV (in the immune compartment only) and as a model for generating bat-specific antibodies against CoV proteins. Efficient validation of infection into bat cells will be used to validate the infectivity of the viruses and generation of bat antibodies will facilitate validation of the best proteins/peptide to elicit an effective immune response.

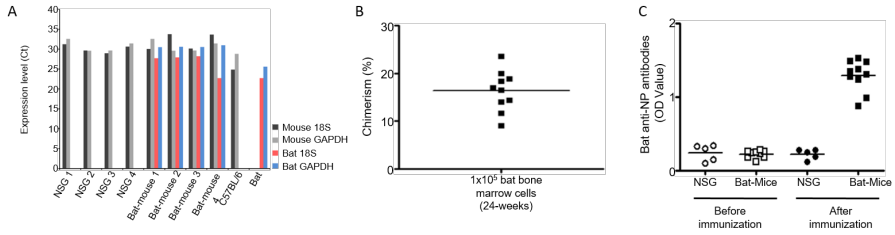

Fig. 9: A) Presence of bat-specific qPCR in reconstituted mice after 12 weeks. B) chimeric ratio of bat-mouse cells in circulation after 24 weeks. C) Specific antibody response to a KLH-tetanus antigen generated by bat-reconstituted mice.

Viral infection models in cave-nectar bat (Duke-NUS): To test and compare the efficacy of the immune modulating approaches above, we will use our cave-nectar bat (*Eonycteris spelaea*)

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breeding colony infected with Melaka virus (family *Reoviridae*) which is known to infect this species[93,94]. We will also use two coronaviruses (SARSr-CoV WIV1 and MERS-CoV in ABSL3. Details of infection, housing, prior infection trials in the facility… Viral loads will be measured by qPCR, titration of produced virus, NGS transcriptomics and nanostring probes added to the immunoprofiling panel. Antibody responses will be measured by LIPS assay. This approach allows us to test our immune-boosting strategies, in a safe and controlled environment, prior to expanding to field-based evaluation. The analytical methods used for the *E. spelaea* colony will be replicated to analyze the experimental infection of *Rhinolophus* in a wild-cave scenario. Additionally, the versatility of the analysis should allow easy application to multiple species of bats

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Targeted Immune Priming (led by Baric, UNC). We have developed novel group 2b SARSr-CoV chimeric S glycoproteins that encode neutralizing domains from phylogenetically distant strains (e.g. Urbani, HKU3, BtCoV 279), which differ by ~25%. The chimeric S programs efficient expression when introduced in the HKU3 backbone full length genome, and elicit protective immunity against multiple group 2b strains. We will develop robust expression systems for SARSr-CoV chimeric S using ectopic expression *in vitro*. Then, we will work with Dr. Ainslie (UNC-Pharmacy) who has developed **novel microparticle delivery systems and dry powders for aerosol release**, and which encapsidate recombinant proteins and adjuvants (innate immune agonists) that will be used for **parallel broadscale immune boosting strategies ± chimeric immungens.** Simultaneously, we will introduce chimeric and wildtype S in raccoon poxvirus (RCN), in collaboration with Dr. Rocke and confirm recombinant protein expression, first *in vitro* and then in the Duke-NUS bat colony, prior to any field trial. The goal of this aim is to develop a suite of reagents to remotely reduce exposure risk in high risk environmental settings.

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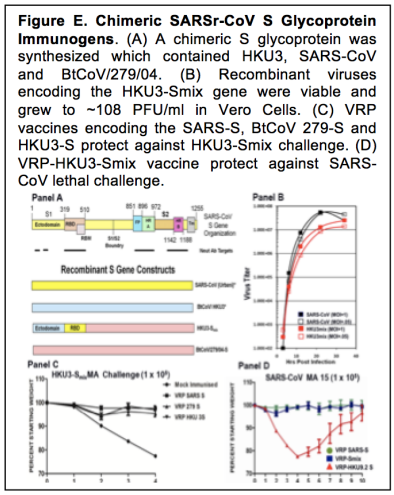

Figure E. Chimeric SARSr-CoV S Glycoprotein Immunogens. (A) A chimeric S glycoprotein was synthesized which contained HKU3, SARS-CoV and BtCoV/279/04. (B) Recombinant viruses encoding the HKU3-Smix gene were viable and grew to ~108 PFU/ml in Vero Cells. (C) VRP vaccines encoding the SARS-S, BtCoV 279-S and HKU3-S protect against HKU3-Smix challenge. (D) VRP-HKU3-Smix vaccine protect against SARS-CoV lethal challenge.

Chimeric SARSr-CoV S Immunogens: CoV evolve quickly by mutation and RNA recombination, the latter provides a strategy to rapidly exchange functional motifs within the S glycoprotein and generate viruses with novel properties in terms of host range and pathogenesis[30,95]. CoV also encode neutralizing epitopes in the amino terminal domain (NTD), RBD and S2 portion of the S glycoprotein[57,96,97], providing a strategy to build chimeric immunogens that induce broadly cross reactive neutralizing antibodies. Given the breadth of SARSr-CoV circulating in

natural settings, chimeric immunogens will be designed to increase the breadth of neutralizing epitopes across the group 2b phylogenetic subgroup[40]. Using synthetic genomes and structure guided design, we fused the NTD of HKU3 (1-319) with the SARS-CoV RBD (320-510) with the remaining BtCoV 279/04 S glycoprotein molecule (511-1255), introduced the chimeric S glycoprotein gene into the HKU3 genome backbone (25% different than SARS-CoV, clade 2 virus) and recovered viable viruses (HKU3-S_{mix}) that could replicate to titers of about 10^8 PFU/ml on Vero cells (Fig. 10). HKU3-Smix is fully neutralized by mAb that specifically target the SARS RBD (data not shown). In parallel, we inserted the $HKU3_{mix}$ S glycoprotein gene into VEE virus replicon vectors (VRP-$S_{chimera}$) and demonstrated that VRP vaccines protect against lethal SARS-CoV challenge and virus growth. In addition, VRP-S_{HKU3} and VRP-S_{279} both protect against $HKU3_{mix}$ challenge and growth *in vivo* (Fig. 9), demonstrating that neutralizing epitopes in the $HKU3_{mix}$ S glycoprotein are appropriately presented and provide broad cross protection against multiple SARSr-CoV strains. In addition to using these immunogens as a targeted broad-based boosting strategy in bats, we will also produce a chimeric SHC014/SARS-CoV/HKU3 S and a SCH014/SARS-CoV/WIV-1 S gene for more focused immune targeting on known high risk strains. In parallel, we will work with the Protein Expression Core at UNC (https://www.med.unc.edu/csb/pep) to produce codon optimized, stabilized and purified prefusion SARS-CoV glycoprotein ectodomains as published previously[17]. Purified recombinant protein will be used by Drs. Rocke and Ainslie for inclusion in delivery matrices (e.g. purified powders, dextran beads, gels – see below) with broadscale immune agonists (adjuvants-Dr. Wang) like poly IC, TLR4 and Sting agonists.

2nd Generation Chimeric S glycoprotein Design and Testing: We will also produce a chimeric SHC014 NTD/SARS-CoV-RBD/HKU3 S C terminal and generate recombinant HKU3 encoding the trimer spike (HKU3-S_{S014}), for more focused immune targeting on known high and low risk strains designated from our experimental and modeling analyses. A second construct will be synthesized with a SHC014 NTD domain, SARS-CoV RBD and WIV-1 C terminal domain (WIV-S_{S014}). After sequence variation, we will evaluate virus growth in Vero and HAE cultures and the ability of SARS RBD monoclonal antibodies (S227, S230, S109) to neutralize chimeric virus infectivity[89,96]. We will also evaluate *in vivo* pathogenesis in C57BL/6 mice and hACE2 transgenic mice. The recombinant HKU3-S_{S014} S genes will be introduced into VRP vectors and sent to Dr. Rocke for insertion into the raccoon poxvirus vaccine vector. Using established techniques, we will characterize S expression and then provide virus vectors to Prof. Wang for immune boosting trials at Duke-NUS, and ultimately if successful in the field (Prof. Shi). We will also synthesize human codon optimized the HKU3-S_{S014}, WIV-S_{S014} and HKU3-S_{mix} chimeric spikes for expression and purification by the UNC proteomics core, producing mg quantities for inclusion in nanoparticle and microparticle carriers in collaboration with Dr. Ainslie. We will produce enough material for *in vivo* testing in mice and in bats. Recombinant HKU3-S_{S014} and WIV-S_{S014} glycoprotein expression will be validated by Western blot and by vaccination of mice, allowing

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us to determine if the recombinant protein elicits neutralizing antibodies that protect against lethal SARS-CoV, HKU3-S_{mix} and SHC014 challenge. In parallel, we will survey the RNAseq data for evidence of complex S glycoprotein gene RNA recombinants in the SARSr-CoV population genetic structure. If present, we will synthesize 2-3 interesting recombinant S genes, insert these genes into SHCO14 or HKU3 genome backbones and VRP and characterize the viability and replicative properties of these viruses in cell culture and in mice and the VRP for S glycoprotein expression and vaccine outcomes. We will produce immunogens and evaluate their ability to protect against infection.

Adjuvant and Immunogen Delivery Vehicles. Dr. Ainslie (UNC) and collaborators have developed the biodegradable polymer acetalated dextran (Ac-DEX) for the delivery of antigens and adjuvants in vaccine applications (Fig. 11). Ac-DEX has distinct advantages over other polymers for vaccine development: 1) synthesis is straightforward and scalable. An FDA-approved water soluble dextran polysaccharide is modified and rendered insoluble in water by a simple one-step modification of its hydroxyl groups with pendant acyclic or cyclic acetal groups[98-100]. Unlike other dextran based vaccine materials, our material is acid sensitive, which has been shown to greatly improve antigen presentation; 2) Ac-DEX microparticles (MPs) can passively target antigen-presenting cells (APCs) based on their size (5-8μm), being phagocytosed by DCs and traffic to the lymph node[101]. Furthermore, APCs have acidic phagosomes that can result in triggered intracellular release due to the acid-sensitivity of Ace-DEX; 3) Ac-DEX MPs and their hydrolytic byproducts are pH-neutral, biocompatible, and safe compared to other commonly used polyesters have acidic hydrolytic byproducts (e.g. lactic and glycolic acid, in the case of PLGA) that damage vaccine components such as protein antigens[102]. The complete hydrolysis of Ac-DEX results in particle breakdown with release of the metabolic side products. 4) Ac-DEX MPs are stable outside the cold-chain. MPs can be stored for at least 3 months at 45°C without any loss of integrity or encapsulated cargo bioactivity[103]. Other common formulations (e.g. liposomes[104], PLGA MPs[103], squalene emulsions [Fluad™ package insert]) have limited shelf-life that requires the cold-chain. Ac-DEX MPs can be aerosolized, or delivered in sprays or gels to bat populations, providing new modalities for zoonotic virus disease control in wildlife populations[98,105]. 5) We have previously encapsulated Poly (I:C)(1), resiquimod[101], and a STING agonist into our novel MPs[106].

As seen in Fig. 10, encapsulation of Poly (I:C) drastically enhances the activity of the TLR agonist. Additionally, encapsulation of adjuvants in MPs drastically enhances the activity of subunit vaccines. We have

Figure F. Particle Delivery Systems. Broadscale immune boosting strategies include (A) Dextran microparticles or Dry nanoparticle powders. (B) Macrophages cultured with either free poly (I:C) or poly (I:C) encapsulated into Ac-DEX MPs produce significant TNFα. (C) Comparison of (left) neutralizing titer and (right) viral load when ferrets are vaccinated with Ac-DEX MPs. Day 0, 28, and 56 (prime, boost, and challenge.)

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displayed better efficacy than state-of-the-art FDA-approved inactivated flu virus (Fluarix) in a ferret model of influenza. The ferret model is the ideal animal model for influenza because of their relatively small size and they possess various clinical features associated with human influenza infection[107]. This formulation used HA with encapsulated STING agonist cyclic [G(3',5')pA(3',5')p](16)

Microparticle Performance Metrics in vitro and in Rodents and Bats: MPs are designed for aerosol delivery due to their relatively effective low aerodynamic diameter[108], their low density microporous nature which allows for efficient aerosol dispersal and deep penetration into the lung, or deposition on the skin for oral uptake by grooming. We will encapsulate Poly (I:C), resiquimod (TLR 7) or other innate immune agonists to enhance type I interferon production in in consultation with Prof Wang. Agonist laden particles will be made separately or in combination with recombinant SARS-CoV chimeric spike proteins, encapsulated into our aerodynamic MPs as well as nanoparticles.

Delivery system development (Rocke, NWHC). We have previously developed, tested and registered oral vaccines and delivery methods to manage disease in free-ranging wildlife including a sylvatic plague vaccine for prairie dogs[24], vaccines against bat rabies[25] and white-nose syndrome (unpubl. data). We have optimized vaccine delivery methods, uptake by the target species and safety in non-target hosts using biomarkers prior to deployment[109]. We will use a similar approach to develop, test and optimize delivery methods to *Rhinolophus* bats in SE Asia. While work on immune modulating agents progresses, we will concurrently develop and test mediums, routes, and methods of delivery to large colonies of bats. We will determine the most feasible and simple method of delivery that achieves high uptake by bats, is safe for humans as well as target and non-target species, and minimizes colony disturbance. Sticky edible gels or pastes that bats groom from themselves and each other have been used previously to deliver pharmaceuticals to bats orally and we are currently testing these for use in rabies vaccine delivery. These may also be useful for delivering immune modulators and recombinant SARSr-CoV spike proteins to *Rhinolophus* bats, but may need to be combined with viral vectors (like poxvirus or adenovirus) or nanoparticles/nanoemulsions that enhance uptake through mucous membranes or transdermally after topical application. *Poxvirus vectors:* Poxviruses are effective viral vectors for delivering vaccines to wildlife [24,110,111], and can replicate safely at high levels in bats after oronasal administration[26]. We have demonstrated proof-of-concept in bats. We tested modified vaccinia Ankara (MVA) and raccoon poxvirus (RCN) vectors for safety and replication in bats using *in vivo* biophotonic imaging[25]. RCN replicated to higher levels in bats than MVA, even via the oral route, and was found to be highly safe for bats (Fig. 12). We used raccoon poxvirus-vectored novel rabies glycoprotein (mosaic or MoG) and demonstrated protective efficacy in bats after oronasal and topical administration[25] (Fig. 13). We are currently developing vaccine delivery for vampire bats in several Latin

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American countries, and vaccines for white-nose syndrome in bats, a devastating disease that has killed millions of insectivorous bats in North America.

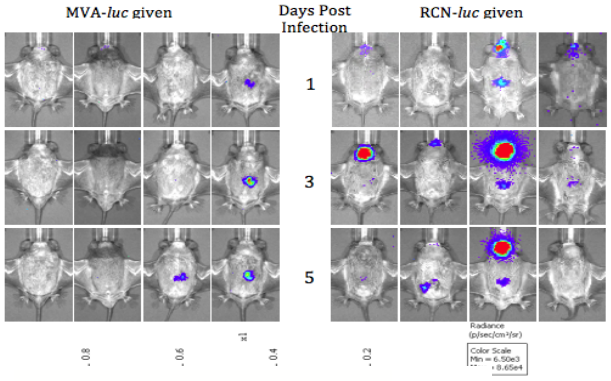

Fig. 12. Luminescence, indicative of viral replication of modified vaccinia Ankara (MVA) and raccoon poxvirus RCN) in the bat Tadarida brasiliensis *on 1, 3 and 5 dpi via the oronasal route.*

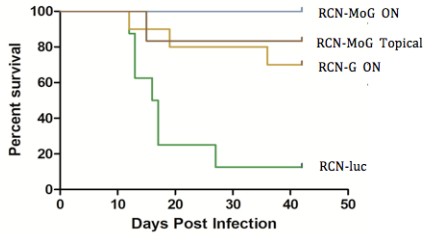

Figure 13. Vaccine efficacy and rabies challenge in Epstesicus fuscus immunized with raccoon poxvirus expressing a mosaic G protein (RCN-MoG) either oronasally (ON) or topically in comparison to RCN expressing typical G protein and RCN expressing luciferase (negative control).

Poxviruses are safe in a wide variety of wild and domestic animals, and allow for large inserts of foreign DNA. We have previously used a raccoon poxvirus vectored vaccine expressing plague antigens that was incorporated into a peanut-butter flavored bait matrix to manage plague caused by *Yersinia pestis* in prairie dogs. We incorporated the biomarker Rhodamine B (RB) into baits to assess uptake by target and non-target species [109,112] (Fig. 14). RB is visible under a UV microscope until the hair grows out (~50 days in prairie dogs). We have since conducted a large field trial (approved by USDA Center for Veterinary Biologics) that demonstrated vaccine efficacy in four species of prairie dogs in seven western states[24]. We used biomarker analysis to assess site- and individual host-specific factors that increased bait consumption including age, weight, and the availability of green vegetation.

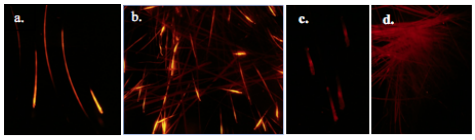

Fig. 14. Prairie dog hair and whisker samples under fluorescence microscope (excitation wavelength: 540 nm, emission wavelength: 625 nm) to determine uptake of baits containing Rhodamine B. a) 20 days after

bait distribution, b) 16 days after bait distribution, c) and d) controls (note natural dull fluorescence).

Transcutaneous delivery: In addition to viral vectors, we will also consider methods to achieve transcutaneous delivery of the immune boosting proteins without the use of live agents. Nanoparticles have been used to increase transcutaneous delivery efficiency[113]. However, the impermeable stratum corneum provides a difficult barrier to breach. Mechanical approaches have been used[113] but are somewhat unethical and impractical for wildlife. We are currently testing poly lactic-co-glycolic acid (PLGA) as a nanoparticle to encapsulate rabies glycoprotein as a method of transcutaneous delivery of vaccine to bats via dendritic cell uptake[114], as has been shown for delivery of TLR agonists and antigens simultaneously to mice[115]. This approach will be competitively trialed against ac-DEX to encapsulate and deliver SARSr-CoV glycoproteins, with and without adjuvants[116], e.g. Matrix M1 (Isconova, Sweden) which has been shown to significantly enhance the immune response in mice to SARS-CoV spike proteins[18]. For efficiency and to reduce costs, initial trials will be conducted in the USA with locally acquired insectivorous big brown bats (*Eptesicus fuscus*) which we have maintained and housed for several experiments at our facility previously[25,26]. We will treat bats via topical application with various test formulations that include the biomarker Rhodamine B (RB), co-house them with untreated bats, and monitor transfer between bats by collecting hair and whiskers for biomarker analysis.

Initial field trials: Bat are not attracted to baits, so delivery in the field is challenging. The high rates of self and mutual grooming observed in bats has previously been exploited to cull vampire bats using poisons like warfarin, applied topically to a small number of bats. Once released, contact and mutual grooming transfers the poison within the colony. We have conducted preliminary biomarker studies in vampire bats in both Mexico and Peru and also in insectivorous bats in Wisconsin. In Peru, we conducted trials with RB-labeled glycerin jelly. Based on capture-recapture data, we estimated a rate of transfer from 1.3 – 2.8 bats for every bat marked. We are analyzing factors associated with rates of transfer, e.g. sex and age of initially treated bats, time of day, to model the rate of vaccination and impact on rabies transmission with different rates of application, prior to actual deployment of vaccine in the field. More recently, we applied RB marked glycerin jelly to the entry of bat houses used by little brown bats (*Myotis lucifugus*). Of 29 bats trapped one week post-application, 59% were positive for biomarker indicating they had eaten the jelly. We will conduct initial trials with each of the delivery vehicles in caves in Wisconsin, targeting local US insectivorous bats. Within one week of application, bats will be trapped at the cave entrance using mist nets or Harp traps and hair will be collected to assess the rate of uptake via biomarker analysis. The bats will be released immediately afterward. The procedures will be tested at several different locations as it will likely take some manipulation to determine appropriate dosages for maximum uptake. After we have determined the most optimal approaches for mass delivery, we will then test

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them on wild bats in our three cave sites in Yunnan Province. Again, biomarker will be used to assess rates of uptake and this data can then be used in modeling studies to help determine the optimal rates of application of immunomodulating agents. Biomarker studies can also be used to assess uptake by non-target species, an important consideration in evaluating safety. Fieldwork will be conducted in collaboration with Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance).

Innovative Aerosol Approach to Bat Inoculation: Once we have confirmed uptake in laboratory studies, we will then assess scalable delivery methods in local caves and hibernacula (using biomarker-labeled mediums but without immunomodulatory substances). In collaboration with Dr. Jerome Unidad of Palo Alto Research Center (PARC), we will develop an innovative aerosol platform technology unique to PARC into a field-deployable prototype for use in cave settings. The technology called Filament Extension Atomization (FEA) can spray fluids with a wide-range of viscosities ranging from 1mPa-s (the viscosity of saliva and most aqueous vaccine formulations) up to 600Pa-s (the viscosity of creams and gels for topical delivery) using a roll-to-roll misting process (https://www.parc.com/services/focus-area/amds/) that results in narrowly-dispersed droplets with tunable sizes from 5-500 microns. FEA technology is compatible with all the formulations of interest to project DEFUSE, including aqueous formulations intended for conventional spraying and the edible gels and creams intended for topical delivery with no limit on bioactive ingredient loading. FEA can then be a universal delivery platform for direct spraying onto bats with the formulation geared towards bio-efficacy.

We will subcontract to PARC to develop a field-deployable FEA prototype, potential form factors for which are shown in Fig. 15F, that can be used in cave settings. PARC will develop the prototype in close collaboration with USGS-NWHC and will conduct the initial trials with them on Wisconsin cave bats. After initial trials, PARC will develop the prototype to a form that will be used for the proof-of-concept demonstration at the test sites in the Kunming bat caves, Yunnan province, China. The field-deployable system will be motion-actuated, and on a timer so that bats will be targeted at fly-in and fly-out but diurnal flying non-target species (e.g. cave swiftlets) can be avoided.

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Deleted: in the form of a field-deployable spray device triggered by timers and movement detectors at critical cave entry points. PARC's Filament Extension Atomization (FEA) (Fig. 15) …

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Deleted: for lab testing, optimize spray conditions for DEFUSE fluids, manipulate fluid formulation for targeted spreading and bioefficacy, and design a prototype field-deployable system. We will initially trial this on captive bats at NWHC, then on Wisconsin cave bats, then at our test sites in Yunnan Province, China. The field-deployable system

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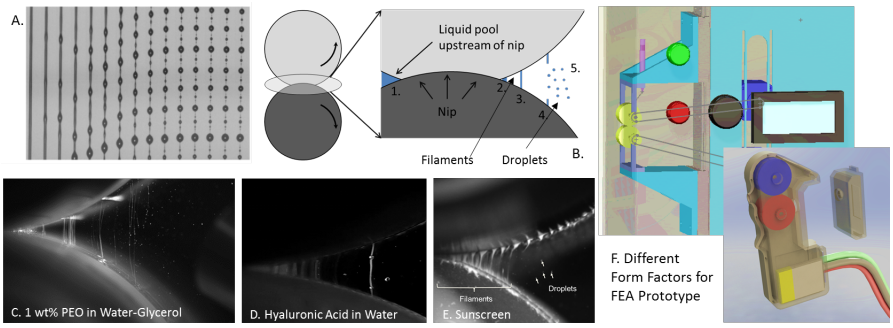

Fig. 15: PARC FEA Technology – A. Beads-on-a-string structures in viscoelastic fluids, B. Parallelization of filament formation and droplet break-up in an FEA roller system, C.-E. Images from high speed videos of representative fluids sprayed with FEA (Polyethylene Oxide in Water-Glycerol, Hyaluronic Acid and Sunscreen), F. Potential form factors of the field-deployable prototype for Project DEFUSE (benchtop, handheld)

Dynamic circulation modeling to optimize deployment strategy. To select amongst various options for immune boosting, priming, and targeting, and multiple delivery options and schedules, we will simulate deployment using a model of viral circulation in cave bat populations. The model will be fit to data from our three-cave test system but designed to be robust to be generalizable to other cases. We will simulate outcomes under a variety of different deployment scenarios to produce conservative estimate of necessary application under real-world conditions. *Fit stochastic viral circulation models to longitudinal sampling data:* We will use longitudinal viral prevalence, mark-recapture estimates of bat populations, radiotelemetry and infrared camera data collected during our field sampling to parameterize and construct models of bat population dynamics and viral circulation in our test caves. We will use a simple but robust stochastic SIR process model with immigration and emigration and flexible, nonlinear contact rates between bats[117]. This model structure can capture a wide range of viral dynamics from intermittent viral outbreaks to regular, endemic circulation with a relatively small number of parameters. We will fit these models to our sampling data using the **partially observable markov process (pomp)** framework[118], allowing estimates of the underlying latent dynamic disease transmission process, accounting for and separating the natural stochasticity of viral circulation and observation error in sampling. We will validate our models via temporal cross-validation: leaving out successive sections on longitudinal time-series from our model fitting to test the model, and by testing the results of a fit from two cave sites on data from a third. *Simulate circulation under a set of plausible deployment scenarios.* Using the top performing sets of immune boosting and targeted immune priming molecules from captive trials, and the delivery media and methods with the greatest uptake rates in cave

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studies, we will use the stochastic SIR model to generate simulations of viral circulation under a series of treatment deployments in our focal study caves. These scenarios will cover a range of plausible intensities, frequencies, and combinations of suppression strategies. They will incorporate uncertainty in the efficacy of each of the treatment strategies. From these simulations, we will estimate the expected degree and time period of suppression of viral circulation and shedding and the uncertainty in this expectations. We will determine the optimal scenario for deployment in our focal study caves. *Test robustness of deployment strategies under broader conditions:* We will use our simulation models to determine best strategies for deployment under a variety of conditions covering likely environments. We anticipate the deployment is likely to occur under (a) highly varied species population and compositions, with uncertain estimates based on rough observations (b) varied uptake and efficacy of immune boosting and targeting molecules due to different environmental conditions, and (c) limited time or resources to deploy treatment. Thus, we will simulate deployment under many potential conditions to determine how optimal deployment differs according to condition, and determine deployment strategies which are conservative and robust to these uncertainties and limitations.

Proof-of-concept deployment of immune modulation molecules in test caves in Yunnan Province, China.

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MANAGEMENT PLAN

- Provide a summary of expertise of the team, including any subcontractors, and key personnel who will be doing the work. Resumes count against the page count.
- Identify a principal investigator for the project.
- Provide a clear description of the team's organization
- Include an organization chart with the following information, as applicable:
  A) Programmatic relationship of team members
  B) Unique capabilities of team members
  C) Task responsibilities of team members
  D) Teaming strategy among the team members
  E) Key personnel with amount of effort to be expended by each during each year
- Provide a detailed plan for coordination including explicit guidelines for interaction among

collaborators/subcontractors of the proposed effort.

- Include risk management approaches.

- Describe any formal teaming agreements that are required to execute this program.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research organization focused on emerging zoonotic diseases. The PI, Dr. Peter Daszak, has 25+ years' experience managing lab, field and modeling research projects on emerging zoonoses. Dr. Daszak will commit 3 months annually to oversee and coordinate all project activities, and lead modeling and analytic work for TA1. Dr. Billy Karesh has 40+ years' experience leading zoonotic and wildlife disease projects, and will commit 1 month annually to manage partnership activities and outreach. Dr. Jon Epstein, with 15 years' experience working emerging bat zoonoses will coordinate animal trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project. Support staff include field surveillance teams, modeling analysts, and consultants based in Yunnan Province, China, to oversee field trials. The EHA team has worked extensively with all other collaborators: Prof. Wang (15+ years); Dr. Shi (15+ years); Prof. Baric (5+ years) and Dr. Rocke (15+ years). **Subcontracts: #1** to Prof. Ralph Baric, UNC, to oversee reverse engineering of SARSr-CoVs, BSL-3 humanized mouse experimental infections, design and testing of immune priming treatments based on recombinant spike proteins. Assisted by senior personnel Dr. Tim Sheahan, Dr. Amy Sims, and support staff; **#2** to Prof. Linfa Wang, Duke NUS, to oversee the immune boosting approach, captive bat experiments, and analyze immunological and virological responses to immune boosting treatments; **#3** to Dr. Zhengli Shi, Wuhan Institute of Virology, to conduct PCR testing, viral discovery and isolation from bat samples collected in China, spike protein binding assays, and some humanized mouse work, as well as experimental trials on *Rhinolophus* bats. Her team will include Dr. Peng Zhou and support staff; **#4** to Dr. Tonie Rocke, USGS National Wildlife Health Center, to refine delivery mechanisms for both immune boosting and immune priming treatments. With a research technician, Dr. Rocke will use a captive colony of bats at NWHC for initial trials, and oversee cave experiments in China; **#5** to Dr. Jerome Unidad, PARC, to develop their innovative aerosol platform into a field-deployable device for large-scale inoculation of the bats. Dr. Unidad will collaborate closely with Dr. Rocke in developing a field-deployable prototype for both initial trials and cave experiments in China.

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Dr. Peter Daszak is President and Chief Scientist of EcoHealth Alliance, a US-based research organization focused on emerging zoonotic diseases. His >300 scientific papers include the first global map of EID hotspots[119,120], estimates of unknown viral diversity[121], predictive models of

virus-host relationships[3], and evidence of the bat origin of SARS-CoV[29] and other emerging viruses [122-125]. He is Chair of the NASEM Forum on Microbial Threats, and is a member of the Executive Committee and the EHA institutional lead for the $130 million USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr. Daszak won the 2000 CSIRO medal for collaborative research.

Prof. Ralph Baric is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Dept. of Epidemiology and Dept. of Microbiology & Immunology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, cross species transmission and pathogenesis. His group has developed a platform strategy to access the potential "pre-epidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (REFS).

Prof. Linfa Wang is Director, Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore. His proven track record in the field includes identifying the bat origin of SARS-CoV, pioneering work on Henipaviruses and many more. His work has shifted from identifying the bat-origin of pathogens to understanding basic bat biology and the mechanisms by which they can endure sustained virus infection. He has received multiple awards including the 2014 Eureka Prize for Research in Infectious Diseases. He currently heads and administers a Singapore National Research Foundation grant on "Learning from bats" for $9.7M SGD. He is an advisory member of …. an Editor of multiple journals and current Editor-in-Chief for the Journal *Virology*.

Dr. Danielle Anderson is the Scientific Director of the Duke-NUS ABSL3 laboratory and is an expert in RNA virus replication. Dr Anderson has extensive experience in both molecular biology and animal models and will lead the animal studies. Dr Anderson has established Zika, Influenza and Reovirus non-human primate (NHP) models in Singapore, using different inoculation routes (such as mosquito inoculation), and has performed trials on over 30 NHPs.

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Dr Aaron Irving is an experienced postdoctoral fellow in the field of innate immunity and viral sensing with expertise focusing on host-pathogen interactions and intrinsic immunity. He oversees multiple projects on bat immune activation within Prof. Linfa Wang's laboratory at Duke-NUS Medical School and has experience in *in vivo* animal infection models.

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Prof. Zhengli Shi: Dr. Shi is the director of the Center for Emerging Infectious Diseases of the Wuhan Institute of Virology, Chinese Academy of Sciences. She got Ph.D training in Virology in Montpellier University II from 1996 to 2000, biosafety training at Australian Animal Health Laboratory in May 2006 and at Lyon P4 in October 2006. She is now in charge of the scientific

activity in BSL3 and BSL4 of the Institute. Her research focuses on viral pathogen discovery through traditional and high-throughput sequencing techniques. She has been studying the wildlife-borne viral pathogens, particularly bat-borne viruses since 2004. Her group has discovered diverse novel viruses/virus antibodies in bats, included SARS-like coronaviruses, adenoviruses, adeno-associated viruses, circoviruses, paramyxoviruses and filoviruses in China. One of her great contributions is to uncover genetically diverse SARS-like coronaviruses in bats with her international collaborators and provide unequivocal evidence that bats are natural reservoir of SARS-CoV by isolation of one strain that is closely related to the SARS-CoV in 2002-3. She has coauthored >100 publications on viral pathogen identification, diagnosis and epidemiology.

Dr. Tonie Rocke is a is a research scientist at the USGS National Wildlife Health Center, the only federal laboratory with the sole mission to manage disease in wild animals. Dr. Rocke's current research is focused on the ecology and management of diseases in wild mammals (e.g. plague, monkeypox, rabies and white-nose syndrome) with the overreaching goal of conservation of threatened and endangered species. She and other colleagues developed an oral recombinant plague vaccine for use in wild rodents. Dr. Rocke lead a large-scale field trial in 7 western states of the U.S. demonstrating that oral vaccination through consumption of vaccine-laden baits could prevent plague in wild prairie dogs, thus reducing the risk of disease for the endangered black-footed ferret, other animals, and possibly humans. Research is ongoing in Dr. Rocke's laboratory to develop a similar oral recombinant vaccine to manage rabies in vampire bats in Latin America and also white-nose syndrome in North American bats, a fungal disease that has killed millions of bats in the last few years in the U.S.

Dr. Jerome Unidad is a Member of Research Staff at the Hardware Systems Laboratory at PARC. His research interests revolve around novel fluid delivery systems (including aerosol delivery) for high viscosity fluids, polymers and biomacromolecules. At PARC, he is the technical lead in developing the FEA spray technology for consumer and biomedical applications, as well as additive manufacturing. He has a PhD in Chemical Engineering, specializing in polymer science and rheology, from the University of Naples "Federico II" in Naples, Italy and was a postdoctoral researcher at Forschungszentrum Juelich in Munich, Germany.

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Dr. Peng Zhou is a

Dr. Xinglou Yang

Dr. Ben Hu

Dr. Kevin Olival is VP for Research at EcoHealth Alliance. His research over the last 15 years has focused on understanding the ecology and evolution of emerging zoonoses, with a focus on

developing analytical tools and modeling approaches to forecast and prioritize the discovery and surveillance of viral zoonoses. This includes a recent large scale analysis identifying host and viral predictors of spillover in mammals [REF, Nature]. He has led several international field teams to investigate bat-borne viruses globally. Dr. Olival is the Modeling and Analytics coordinator for the USAID PREDICT-2 project; co-PI on an NIH-NIAID project to investigate CoVs in China; and PI on recent DTRA-CBEP grant to characterize CoVs from bats in Western Asia.

Please follow the same format and create Bios for all other personnel with Ph.D and higher. Peter Daszak will then work out how much space we have and decide who to include…

CAPABILITIES

- Describe organizational experience in relevant subject area(s), existing intellectual property, specialized facilities, and any Government-furnished materials or information.

- Discuss any work in closely related research areas and previous accomplishments.

(The following information was taken from the 'Goals and Impact' section of the abstract we submitted).

The SARSr-CoV-bat system, and immune modulation focus: Our group's 15 yrs work on the SARSr-CoV – *Rhinolophus* bat system in China has identified and isolated SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV (e.g. SCH014 & WIV-1). We have shown they bind and replicate efficiently in primary human lung airway cells and that chimeras with SARSr-CoV spike proteins in a SARS-CoV backbone cause SARS-like illness in humanized mice, with clinical signs that are not reduced by SARS monoclonal therapy or vaccination. We have identified a single cave site in Yunnan Province where bat SARSr-CoVs contain all the genetic components of epidemic SARS-CoV *(7,8,9)*. We have now shown that people living up to 6 kilometers from this cave have SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as **a clear-and-present danger of a new SARS-like pandemic**. Our work on bat immunology suggests that bats' unique flying ability has led to downregulated innate immune genes, and their ability to coexist with viruses such as SARSr-CoVs, henipa- and filoviruses that are lethal in many other mammals *(3)*. We have identified bat-specific constitutively expressed bat interferon, a dampened STING-interferon production pathway *(4, 5)*, and have identified a series of other innate immunity factors that are dampened in bats *(6)*.

STATEMENT OF WORK

- Provide a detailed task breakdown, citing specific tasks and their connection to the interim milestones and program metrics.

- Each phase of the program (Phase I base and Phase II option) should be separately defined in the SOW and each task should be identified by TA (1 or 2).

NOTE: The SOW must not include proprietary information.

- For each task/subtask, provide:
    - A detailed description of the approach to be taken to accomplish each defined task/subtask.
    - Identification of the primary organization responsible for task execution (prime contractor, subcontractor(s), consultant(s), by name).
    - A measurable milestone, i.e., a deliverable, demonstration, or other event/activity that marks task completion. Include quantitative metrics.
    - A definition of all deliverables (e.g. data, reports, software) to be provided to the Government in support of the proposed tasks/subtasks.

Phase I:

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TA-01 Task 1.1 Construct species distribution models to predict viral spillover risk in cave bats in South and Southeast Asia
Sub-task 1.1.1.;lkj;lkj;klj
Sub-task 1.1.2.;lj;lkj;lkj
Deliverables: models capable of …..

TA-01 Task 2.5: Field studies to collect tolerant reservoir species. (EcoHealth Alliance, William Karesh).

Sub-Task 2.5.1. Apply for and obtain IACUC approval and appropriate wildlife permits in Bangladesh for sample collection. Collection of blood and urogenital, oropharyngeal and rectal swab

specimens from targeted bat, rodent and non-human primate species from Bangladesh (n = 1000 specimens). Collection of wing-punch dermal tissue biopsies from bats (n = 300).

Sub-Task 2.5.2. Field work is to be conducted by a trained field team using ethical, nondestructive capture, restraint, and sample collection techniques (with IACUC and local government approval). Samples are to be preserved in RNA later (or other preservative) to maintain cellular integrity and frozen at the point of collection using a liquid nitrogen dry shipper and maintained in -80°C. All samples are to be shipped with appropriate government permission and export permits.

Deliverables: 1000 field specimens (whole blood, nasal/rectal swabs) collected from reservoir bats, rodents and non-human primates which have been obtained with all proper permits and permissions are appropriately shipped for further analysis.

TA1:

Task 1.1

Sub-task 1.1.1. Models to predict bat community in caves across S. and SE Asia.

Organization leading task: EcoHealth Alliance

Sub-task 1.1.2. Models to predict presence of viruses with zoonotic potential in bats across S. and SE Asia.

Progress Metrics:

- Joint species distribution model fit for Asian Bats
- Cave-level predictions of bat community composition
- Linear predictions of viral diversity in cave populations
- JSDM predictions of viral diversity in cave populations
- Prediction validations

Deliverable(s):

- Deployable spatial model software of bat community composition
- Deployable spatial model software of viral diversity in bat cave populations

Progress Metrics:

- Joint species distribution model fit for Asian Bats
- Cave-level predictions of bat community composition
- Linear predictions of viral diversity in cave populations
- JSDM predictions of viral diversity in cave populations
- Prediction validations

Deliverable(s):

- Deployable spatial model software of bat community composition
- Deployable spatial model software of viral diversity in bat cave populations

Subtask 1.1.3: Develop prototype app for the warfighter
Description and execution:
Preliminary Data:
Organization leading task: EcoHealth Alliance
Progress Metrics: Development of fully functional and user-friendly application. Use of application in the field.
Deliverables:

Task 1.2: Determining baseline risk of SARSr-CoV emergence in Yunnan, China
Subtask 1.2.1. Longitudinal sampling of bats to determine virus prevalence and diversity in Yunnan cave sites.
Subtask 1.2.2. Analyzing ability of CoVs to infect and emerge in people

***(TA1)* Subtask 5: Assay SARr-CoV quasispecies for spillover potential via assays for binding, cell entry, and pathogenesis in mouse models.**

Organization leading task: University of North Carolina

Progress Metrics: Not sure how to do this.

Deliverable(s):

1. *Methods to Produce Synthetic SARSr-CoV Virus Molecular Clones and Reverse Genetics.*
    a. ***Preliminary Data**:* Molecular Clones for SARSr-CoV WIV1, WIV16, SHC014 and HKU3-SRBD exist. We have demonstrated in the preliminary data that these reagents are already available.
    b. ***Target Goals**:* We will generate molecular constructs for 20+ chimeric SARSr-CoV encoding different S glycoprotein genes/yr
    c. **Target Goals***: We will generate 2-5 full length molecular clones of SARSr-CoV.*

2. *Methods of Recombinant virus Recovery and Characterization*
    a. **Preliminary Data***: Demonstrated recovery recombinant chimeric SARSr-CoV WIV1, WIV16, SHC014, HKU3-SRBD, including full length recombinant viruses of WIV1, WIV16, SHC014 and HKU3-SRBD.*
    b. *Target Goals:* We will isolate 20+ chimeric SARSr-CoV encoding novel S

glycoprotein genes

c. *Target Goals:* We will isolate 2-5 full length SARSr-CoV/year/
    i. **Key Deliverables for Program-wide Success***: These two key reagents position us for immediate testing of the antiviral effects of* **broadscale immune boosting molecules** +/- immunogens on virus growth *in vitro* and *in vivo*, and on virus levels in models of chronic SARS-CoV infection in mice.

3. *Virus Phenotyping: Receptor Interactions and In Vitro Growth.*
    a. ***Preliminary Data****: Cell lines encoding bat, human, civet and mouse ACE2 receptors exist and have been validated. We have demonstrated the use of primary human airway epithelial cultures to characterize SARSr-CoV pre-epidemic potential.*
    b. ***Target Goals****: We will characterize SARSr-CoV recombinant virus growth in Vero cells, nonpermissive cells encoding the civet, bat and human ACE2 receptors.*

4. *Virus Pathogenic Potential in Humans:*
    a. *Preliminary Data: We also have transgenic human ACE2 mouse models to compare the pathogenic potential of SARSr-CoV*
    b. *Target Goals: We will evaluate SARSr-CoV pathogenic outcomes in hACE2 transgenic mice.*

5. *Virus Antigenic Variation:*
    a. *Preliminary Data: We have robust panels of broadly cross reactive human monoclonal antibodies against SARS and related viruses and mouse models to evaluate protection against SARSr-CoV replication and pathogenesis.*
    b. *We will evaluate SARS-vaccine performance against a select subset of SARSr-CoV (10), chosen based on the overall percent of antigenic variation, coupled with distribution across the S glycoprotein structure.*

6. *Low Abundant High Consequence Sequence Variants:*
    a. *We will identify the presence of low abundant, high risk SARSr-CoV, based on deep sequencing data*

7. *Proteolytic Processing and Pre-epidemic Potential.*
    a. *We will evaluate the role of proteolytic cleavage site variation on SARSr-CoV cross species transmission and pathogenesis in vivo.*

(TA1) **Subtask 4: Build models to predict viral species spillover potential and evoluation**

Organization leading task: EcoHealth Alliance

Description and execution:

Progress Metrics:

- *Development of prior-based pathogenicity predictions and sequence testing guidance*
- *Model fits from initial rounds of viral characterization*
- *Model fits from secondary rounds of viral characterization*
- *Predictions of spillover probability of sequenced viral QS*
- *Deployable predictive model*

Deliverable(s):

- Fit models as reproducible, deployable software providing virus spillover potential predictions and uncertainties based on input of host species and viral sequence data
- Ranking of potential pathogenicity of virus QS from both Task X sampling and previous data.

***(TA2)* Task 5: Trial experimental approaches aimed towards 'Broadscale Immune Boosting' using experimental bat colonies**

TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.

Organization leading task: Wuhan Institute of Virology, Duke-NUS

***(TA2)* Task 6: Trial experimental approaches aimed towards 'Immune Targeting' using experimental bat colonies**

Organization leading task: University of North Carolina

Progress Metrics:

Deliverable(s):

1. ***Chimeric S-Glycoprotein Antigen Design, Recovery and Phenotyping for Immune Boosting***.
    a. **Preliminary Data***: Demonstrated recovery recombinant chimeric HKU3-S_{mix}, demonstrating preservation of entry functions in the chimeric spike. Neutralizing*

epitopes and in vivo pathogenesis phenotypes were also preserved. Chimeric Spikes are biologically functional.

b. ***Target Goals****:* We will isolate chimeric HKU3-S_{S014} S and WIV-S_{S014} genes, chimeric viruses and express the S glycoprotein from VRP and raccoon poxvirus expression vectors.

c. ***Target Goals:*** We will synthesize 2-3 chimeric S glycoproteins, recover recombinant viruses derived from natural recombinants in the population genetic structure of SARSr-CoV. We will also characterized recombinant protein expression from VRP and raccoon poxviruses.

d. ***Target Goals****:* We produce sufficient recombinant HKU3-S_{S014}, WIV-S_{S014} and HKU3-S_{mix} S glycoproteins for inclusion in nanoparticle and microparticle delivery vehicles.

   i. **Key Deliverables for Program-wide Success***: These two key reagents position us for immediate testing of the antiviral effects of* **broadscale immune boosting molecules** +/- immunogens.

2. ***Virus Phenotyping: Receptor Interactions and Growth in vitro and in vivo****.*

   a. ***Preliminary Data****: We have well developed metrics for evaluating chimeric S glycoprotein function in the context of whole virus, neutralization phenotypes and expression as recombinant proteins vaccines for testing in mice.*

   b. ***Target Goals****: Demonstrate chimeric S function in the context of virus infection in Vero and HAE cells and susceptibility to neutralizing antibodies targeted the SARS RBD.*

   c. ***Target Goals****: Evaluate chimeric virus pathogenesis in hACE2 transgenic mice and the ability of VRP vaccines encoding chimeric spikes to elicit protective immunity against lethal SARS-CoV, HKU3-S_{mix} and SCH014 challenge.*

3. ***Production of Agonist (TLR4, dsRNA, Sting) and Chimeric S glycoprotein Nanoparticle and Microparticle Suspensions for in vivo studies***

   a. ***Preliminary Data****: Robust preliminary data exists on the production and immunogenicity of nanoparticle and microparticle delivery systems.*

   b. ***Target Goals****: Produce nanoparticle and microparticle delivery systems encoding agonists, coupled with in vitro testing in vitro in bat and in other reporter cells, mice and bats.*

   c. ***Target Goals****: Inclusion of chimeric recombinant proteins and agonists in nanoparticle and microparticle delivery vehicles, coupled with testing in vitro and in vivo in mice and bats.*

   d. ***Target Goals***: Perform *in vivo* testing in collaboration with Dr. Shi and Dr. Wang.

Task 7: Develop and assess delivery methods to bats for immune boosting and priming molecules

Organization leading task: USGS National Wildlife Health Center
Participating organizations: Palo Alto Research Center (PARC)

Progress Metrics: Still not sure what format you want this in?

Deliverable(s):

1. Poxvirus construct expressing optimal SARS/CoV spike protein for immunizing bats

a. Genetically insert SARS/CoV spike proteins into raccoon poxvirus and confirm antigen expression

b. Conduct laboratory studies to confirm serologic conversion, first in mice (UNC) and then in bats (NWHC)

c. Master seed production of viral stocks for use in later field trials

2. Mediums/vehicles and methods to deliver immunomodulatory agents to bats.

a/ Determine appropriate medium (e.g. glycerin jelly or other viscous substance) for delivering virally vectored vaccines and nanoparticles to bats

b. Assess minimum dosage required for adequate uptake by bats after topical application

c. Determine appropriate delivery methods to apply appropriate dosages in conjunction with PARC, first in laboratory settings, and then in local field sites

3. Prototype system for automatic, mass delivery of immunomodulatory substances to bats (in collaboration with PARC)

a. Conduct biomarker studies to validate application methods in bats, first in local field sites and then at sites in China

b.. Conduct field trial in China with prototype delivery method using selected immunomodulatory substances deemed most useful for bats

4. Data on uptake in insectivorous bats.

a. Provide data on biomarker uptake in insectivorous bats for use in modeling studies

5. Annual reports, manuscripts, presentations.

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SCHEDULE AND MILESTONES

- Provide a detailed schedule showing tasks (task name, duration, work breakdown structure element as applicable, performing organization), milestones, and the interrelationships among tasks.

  NOTE: Task structure must be consistent with that in the SOW.

- Measurable milestones should be clearly articulated and defined in time relative to the start of the project.

PREEMPT TRANSITION PLAN

- Indicate the types of partners (e.g. government, private industry, non-profit)
- Submit a timeline with incremental milestones toward successful engagement. NOTE: begin transition activities during the early stages of the program (Phase I).
- Describe any potential DARPA roles.

Project DEFUSE partners come from academic, government, private industry, private non-profit institutions and will develop a coherent transition plan for research findings, data and any technology developed in this work.

PARC as a private industry partner (large business) is a fully-owned subsidiary of Xerox Corporation and is committed to commercializing the FEA technology through IP licensing for different applications spaces to different commercial partners. In the context of project DEFUSE, PARC has been and will continue to engage potential licensees (OEMs) in the biotechnology and biomedical fields for eventual transitioning of targeted delivery technology that might result in the project. PARC already has existing networks of business relations in the biotechnology and biomedical space, both large companies (Fortune 500, Fortune 1000) and small businesses and start-ups who could be transition partners for FEA as a wide-scale, large-area drug delivery device. In addition, in collaboration with our extended network of DEFUSE partners and with DARPA, we will further identify existing government needs for our delivery technology, particularly in wildlife health management (in collaboration with EHA and USGS-NWHC) as well as in suppression of emerging threats (in collaboration with government agencies such as the CDC). PARC will leverage this knowledge in developing a needs-based commercialization plan with potential partners.

Commented [PD47]: Description from the BAA:

PREEMPT Transition Plan
Proposers must include a PREEMPT Technology Transition Plan. Proposers must indicate the types of partners (e.g. government, private industry, non-profit) they plan to pursue and submit a timeline with incremental milestones toward successful engagement. Proposers should begin transition activities during the early stages of the program (Phase I). Awardees must include
DARPA in the development of transition relationships. If the transition plan includes a start-up company, a business development strategy must be included as well. The extent by which the proposed intellectual property (IP) rights will impede the Government's ability to transition the technology will be considered in the proposal evaluation.

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PREEMPT RISK MITIGATION PLAN

- Provide the following:
    - An assessment of potential risks to public health, agriculture, plants, animals, the environment, and national security.
    - Guidelines the proposer will follow to ensure maximal biosafety and biosecurity.
    - A communication plan that addresses content, timing, and the extent of distribution of potentially sensitive dual-use information. The plan must also address how input from DARPA, other government, and community stakeholders will be taken into account in decisions regarding communication and publication of potentially sensitive dual-use information.

ETHICAL, LEGAL, SOCIETAL IMPLICATIONS

- Address potential ethical, legal, and societal implications of the proposed technology.

BIBLIOGRAPHY

A) Brief Bibliography (no page limit indicated – can be published/unpublished)
This and next part don't count toward 36 page limit

RELEVANT PAPERS

B) Up to 3 relevant papers attached (optional)
Propose:

- Ge et al. Nature
- Menacherry et al.
- Zhou et al. SADS-CoV

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Fwd: letter for Jonathan to sign

Rocke, Tonie E <trocke@usgs.gov>

Wed 3/21/2018 4:42 PM

To: Luke Hamel <hamel@ecohealthalliance.org>

Here's the letter you need. -Tonie

---------- Forwarded message ----------
From: **Schroeder, Rebecca** <rschroeder@usgs.gov>
Date: Wed, Mar 21, 2018 at 4:09 PM
Subject: Re: letter for Jonathan to sign
To: "Rocke, Tonie" <trocke@usgs.gov>

Here you go.

Becky Schroeder
Executive Assistant to the Director's Office
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
Phone: (608) 270-2402
Email: RSchroeder@usgs.gov

On Wed, Mar 21, 2018 at 1:07 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Hi Rebecca: Can you put this draft letter on our letterhead, add Jonathan's credentials as he likes to use, and get his signature? If you send the signed letter back to me, I'll pass it on. Thanks much! -Tonie
>
> --
> Tonie E. Rocke
> USGS National Wildlife Health Center
> 6006 Schroeder Rd.
> Madison, WI 53711
> 608-270-2451
> trocke@usgs.gov

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
National Wildlife Health Center
6006 Schroeder Road
Madison, Wisconsin 53711-6223

20 March 2018

Dr. Peter Daszak
President
EcoHealth Alliance
460 West 34th Street
New York, NY 10001

Dear Dr. Daszak:

In regards to the DARPA BAA, PREventing EMerging Pathogenic Threats (PREEMPT), Ref. #: HR001118S0017-PREEMPT-PA-001, PROJECT DEFUSE, the National Wildlife Health Center (NWHC) will be pleased to collaborate with EcoHealth Alliance (EHA) in the implementation of the DEFUSE project should the team be chosen by DARPA to conduct the work.

In our discussions, we have agreed to participate in activities that aim to defuse the potential for spillover and emergence of novel bat-origin high-impact SARS-related coronaviruses from bats to people. Our assistance would include developing and implementing a delivery method for the immune boosting and priming molecules that serve to defuse the potential of disease spillover.

I would also like to confirm that the NWHC has the statutory authority to propose to Government solicitations, such as PREEMPT, under 31 U.S.C 1535A Economy Act. NWHC is a world leader in the development of oral vaccines and delivery methods to manage disease in bats and other free-ranging wildlife. For this reason, and given the 15+ years of collaborative research between the NWHC and EHA, I believe that the NWHC is uniquely capable of addressing the technical challenges listed under PREEMPT.

Furthermore, the NWHC, to the best of my knowledge, does not have any conflict of interest with EcoHealth Alliance, nor its collaborators on this project.

On behalf of the NWHC, please list us as a partner in your DEFUSE project proposal. I look forward to working on DEFUSE with EcoHealth Alliance and its partners in this critically important endeavor.

Sincerely,

Jonathan Sleeman

Jonathan Sleeman, MA, VetMB, Dipl. ACZM, Dipl. ECZM, MRCVS
Center Director

Re: DARPA PRE-EMPT

Rocke, Tonie E <trocke@usgs.gov>

Tue 3/20/2018 4:20 PM

To: Anna Willoughby <willoughby@ecohealthalliance.org>

Hi Anna: Should I wait for Peter's updated draft before I make revisions? Also, attached is a file containing bios for both Rachel Abbott and myself. Please let me know if you think they are sufficient. Best -Tonie

On Tue, Mar 20, 2018 at 4:15 PM, Anna Willoughby <willoughby@ecohealthalliance.org> wrote:

> Dear all,
>
> Please find the NWHC section of the proposal. Peter is currently working on an updated draft, but this should be sufficient for composing your budget and particular task. Please let me know if you have any questions. If needed, I am also attaching the original BAA for the program, with PARC's focus being TA2.
>
> When can we expect the revised budget and scope of work?
>
> Best,
> Anna
>
> On Fri, Mar 16, 2018 at 3:12 PM, Anna Willoughby <willoughby@ecohealthalliance.org> wrote:
>
> > Thanks for these details, Jerome. Attached are my notes from the call. Action items include:
> >
> > *- Jerome to send more detailed scope of work with paragraphs and revised budget by early next week*
> > *- EHA will send PARC the NWHC section of the proposal on Monday*
> > *- EHA will send the format of letter of support for PARC*
> > *- EHA to follow up with Kateri with requested information*
> >
> > For your question on collaborating with other institutes, it is likely that all organizations involved may have insight into the aerosol-bat interaction. I believe this topic would be covered during the Annual Meeting between all partners, as well as during relevant cross-partner trips, in addition to monthly conference calls.
> >
> > Please let us know if you have further questions.
> >
> > Best,
> > Anna

On Fri, Mar 16, 2018 at 2:57 PM, <Jerome.Unidad@parc.com> wrote:

> An additional point for Peter, Tonie (and everyone),
>
> For the spray technology, refinement of the details with respect to aerosol-bat interaction (i.e. the preliminary field testing to see how bats react to the aerosol) and eventual field-deployment in China, will the technical lead for coordinating this segment of the project be USGS – National Wildlife Center? Or should we also expect to work/coordinate with other institutes who would give feedback and insights on how this works?
>
> Thanks. This is just for our information.
>
> Best,
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company
>
> ---
>
> **From:** Unidad, Jerome <Jerome.Unidad@parc.com>
> **Sent:** Friday, March 16, 2018 11:52 AM
> **To:** 'William B. Karesh' <karesh@ecohealthalliance.org>; 'Peter Daszak' <daszak@ecohealthalliance.org>
> **Cc:** 'Luke Hamel' <hamel@ecohealthalliance.org>; 'Anna Willoughby' <willoughby@ecohealthalliance.org>; 'Alison Andre' <andre@ecohealthalliance.org>; 'Amanda Andre' <amanda.andre@ecohealthalliance.org>; 'Rocke, Tonie' <trocke@usgs.gov>; Paul, Kateri <Kateri.Paul@parc.com> <Kateri.Paul@parc.com>
> **Subject:** RE: DARPA PRE-EMPT
>
> Peter and team,
>
> I'm currently working on putting together a revised budget and equivalent statement of work (tasks breakdown) for PARC's involvement with the project. You can expect this about early next week – approximately Monday. Officially, for the submission, our capture manager, Kateri Paul, who takes care of the other things would need the following things from your equivalent to facilitate our parts of the submission.

1. Request for Proposal that we can respond to with what they need for their package to DARPA
2. Start date of the proposed effort
3. Contract or a Grant/Other Transaction

Once we have finalized the scope of work and the budget, Kateri will be in touch for these other aspects. Her contact information can be found below.

Kateri E. Paul

Capture Manager, Public Sector

Global Business Development

Palo Alto Research Center (PARC)

3333 Coyote Hill Road

Palo Alto, CA 94304

Kateri.Paul@parc.com

650-812-4821 (desk)

617-596-2023 (mobile)

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Unidad, Jerome <Jerome.Unidad@parc.com>
Sent: Thursday, March 15, 2018 3:33 PM
To: 'Rocke, Tonie' <trocke@usgs.gov>; William B. Karesh <karesh@ecohealthalliance.org>; Johnson, David <David.Johnson@parc.com> <David.Johnson@parc.com>
Cc: Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: RE: DARPA PRE-EMPT

Dear all,

10AM-11AM PST (12PM-1PM CT, 1PM-2PM ET) should work for us. I shall setup a WebEx meeting for this, given the number of participants.

Let me know if this timeslot will work.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 2:39 PM
To: William B. Karesh <karesh@ecohealthalliance.org>
Cc: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I assume that is ET? -T

On Thu, Mar 15, 2018 at 4:14 PM, William B. Karesh <karesh@ecohealthalliance.org> wrote:

> Tonie and Jerome,
>
> We would still like to speak. Anytime on Friday between 11:00 AM and 2:00 PM would be great.
>
> BK

William B. Karesh, D.V.M

Executive Vice President for Health and Policy

EcoHealth Alliance

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Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

> On Mar 15, 2018, at 4:55 PM, Rocke, Tonie <trocke@usgs.gov> wrote:
>
> Hi all: Since we didn't hear back from EcoHealth Alliance, Jerome and I went ahead with a short call we had been planning anyway regarding some technical details. I told him our concerns about the proposed budget and we

think we have a pretty good plan to reduce the scope of work to the funds we have available. PARC is very unique in developing this technology and their technology fits very well with other work I am doing, so we both feel pretty confident we can work something out. If you still wish to have a discussion among all of us, we can schedule that for tomorrow, as I believe Jerome had another meeting to run off to for the rest of the day. I'm available the rest of the day if you wish to chat about this in person. Best -Tonie

On Thu, Mar 15, 2018 at 3:42 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

> Actually – can we do a phone call – I'll be driving. 5.15pm would be perfect (NYC time), Today Thursday.
>
> Is that possible?
>
> Our call in line is: 1-719-785-9461
>
> Passcode: 9784#
>
> Cheers,
>
> Peter
>
> **Peter Daszak**
>
> *President*
>
> EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4474

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@PeterDaszak

@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Jerome.Unidad@parc.com [mailto:Jerome.Unidad@parc.com]
Sent: Thursday, March 15, 2018 4:23 PM
To: trocke@usgs.gov
Cc: William B. Karesh; Peter Daszak; Luke Hamel
Subject: RE: DARPA PRE-EMPT

I can setup a WebEx quickly if we will have multiple parties.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 1:22 PM
To: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Daszak Peter

<daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I'm available as well. Billy, do you have a call in number? -Tonie

On Thu, Mar 15, 2018 at 3:20 PM, <Jerome.Unidad@parc.com> wrote:

> Dear all,
>
> Sorry for the late response – yes, I will be available for a phone call now. Up to 2PM.
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company
>
> ---
>
> **From:** William B. Karesh [mailto:karesh@ecohealthalliance.org]
> **Sent:** Thursday, March 15, 2018 12:49 PM
> **To:** Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
> **Cc:** Rocke, Tonie <trocke@usgs.gov>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
> **Subject:** DARPA PRE-EMPT
>
> Dear Dr. Unidad,
>
> Thanks for your quick responses to Dr. Rocke. Would you be available for a short call with Dr. Daszak, Dr. Rocke and me this afternoon or Friday.
>
> We're on tight timeline so we thought a phone call might be save quite a bit of time.
>
> Thanks in advance,

Billy

William B. Karesh, D.V.M

Executive Vice President for Health and Policy

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--

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--

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Anna Willoughby

Research Assistant

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--

Anna Willoughby

Research Assistant

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> ***EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.***

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

Dr. Tonie Rocke, Ph.D. is a research scientist at the USGS National Wildlife Health Center, the only federal laboratory with the sole mission to manage disease in wild animals. Dr. Rocke's current research is focused on the ecology and management of diseases in wild mammals (e.g. plague, monkeypox, rabies and white-nose syndrome) with the overreaching goal of conservation of threatened and endangered species. She and other colleagues developed an oral recombinant plague vaccine for use in wild rodents. Dr. Rocke lead a large-scale field trial in 7 western states of the U.S. demonstrating that oral vaccination through consumption of vaccine-laden baits could prevent plague in wild prairie dogs, thus reducing the risk of disease for the endangered black-footed ferret, other animals, and possibly humans. Research is ongoing in Dr. Rocke's laboratory to develop a similar oral recombinant vaccine to manage rabies in vampire bats in Latin America and also white-nose syndrome in North American bats, a fungal disease that has killed millions of bats in the last few years in the U.S.
For more information, see: https://www.researchgate.net/profile/Tonie_Rocke/publications and http://www.nwhc.usgs.gov/

Dr. Rachel Abbott, DVM, M.S. has been managing field and laboratory projects as a part of Dr. Rocke's team at the USGS National Wildlife Health Center for the last 6 years. She has played a key role in plague vaccine studies and more recent work on white nose syndrome in bats. Dr. Abbott designs studies, coordinates animal work, oversees technical help, analyzes data, and prepares manuscripts and written reports. For more information, see: https://www.researchgate.net/profile/Rachel_Abbott/publications.

revised budget

Rocke, Tonie E <trocke@usgs.gov>

Tue 3/20/2018 3:57 PM

To: Anna Willoughby <willoughby@ecohealthalliance.org>

Ok Anna, I think I have this broken down as far as I can. Please review and let me know. Best -Tonie

--

Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

TRAVEL

Trip #:	1	Y1	Location:	Arlington, VA		
Purpose:	DARPA Kickoff Meeting					
Days	**# of People**		**Airfare**		**Per Diem**	**Lodging**
1.75	1		$333.00		$172.50	$500.00
Itemized Expenses for "Other"						
	Description		**Amount**			
	parking		**20**			
Transportation to/from airport and in Arlington			$100.00			
		Total:	$120.00			

Trip #:	2	Y1	Location:	Kunning, China		
Purpose:	Site Visit					
Days	**# of People**		**Airfare**		**Per Diem**	**Lodging**
7	1		$1,370.00		$1,035.00	$1,029.00
Itemized Expenses for "Other"						
	Description		**Amount**			
	parking		80			
Transportation to/from airport and in Arlington			$100.00			

Trip #:	3	Y1 option	Location:	Kunning, China		
Purpose:	Deployment Visit					
Days	**# of People**		**Airfare**		**Per Diem**	**Lodging**
7	1		$1,370.00		$1,035.00	$1,029.00
Itemized Expenses for "Other"						
	Description		**Amount**			
	parking		80			
Transportation to/from airport and in Arlington			$100.00			
		Total:	$100.00			

Trip #:	4	Y1	Location:	New York, NY		
Purpose:	Annual Meeting					
Days	**# of People**		**Airfare**		**Per Diem**	**Lodging**
3	2		$666.00		$518.00	$2,328.00
Itemized Expenses for "Other"						
	Description		**Amount**			
	parking		40,00			
transportation			$100.00			
		Total:	$140.00			

Trip #:	5	Y2	Location:	Wuhan, China		
Purpose:	Annual Meeting					
Days	**# of People**		**Airfare**		**Per Diem**	**Lodging**
3	1		$6,861.00		$575.00	$588.00
Itemized Expenses for "Other"						
	Description		**Amount**			
	parking		40			
Transportation to/from airport			$100.00			

		Total:	$140.00			
Trip #:	6	Y3	**Location:**	New York, NY		
Purpose:	Annual Meeting					
Days	**# of People**		**Airfare**		**Per Diem**	**Lodging**
3	2		$666.00		$518.00	$2,328.00
Itemized Expenses for "Other"						
	Description		**Amount**			
parking			40			
Transportation to/from airport			$100.00			
		Total:	$140.00			
Trip #:	7	Y3.5	**Location:**	New York, NY		
Purpose:	Annual Meeting					
Days	**# of People**		**Airfare**		**Per Diem**	**Lodging**
4	2		$666.00		$518.00	$2,328.00
Itemized Expenses for "Other"						
	Description		**Amount**			
	parking		40			
Transportation to/from airport			$100.00			
		Total:	$140.00			
Trip #:	8	Y1	**Location:**	Upper Peninsula Michagan		
Purpose:	Annual Meeting					
Days	**# of People**		**Airfare**		**Per Diem**	**Lodging**
4	3		$0.00		$459.00	$837.00
Itemized Expenses for "Other"						
	Description		**Amount**			
	gas		120			
	govt car use		$468.00			
		Total:	$588.00			
Trip #:	9	Y2	**Location:**	Upper Peninsula Michagan		
Purpose:	Annual Meeting					
Days	**# of People**		**Airfare**		**Per Diem**	**Lodging**
4	3		$0.00		$459.00	$837.00
Itemized Expenses for "Other"						
	Description		**Amount**			
	gas		120			
	govt car use		$468.00			
		Total:	$588.00			
Trip #:	10	Y3	**Location:**	Upper Peninsula Michagan		
Purpose:	Annual Meeting					
Days	**# of People**		**Airfare**		**Per Diem**	**Lodging**
4	3		$0.00		$459.00	$837.00
Itemized Expenses for "Other"						
	Description		**Amount**			

	gas		120
	govt car use		$468.00
		Total:	$588.00

Contract Period	
Base Period	
Other	**Total**
$120.00	$1,125.50

Contract Period	
Base Period	
Other	**Total**
$180.00	$3,614.00

Contract Period	
Option I	
Other	**Total**
$180.00	$3,614.00

Contract Period	
Base Period	
Other	**Total**
$140.00	$3,652.00

Contract Period	
Base Period	
Other	**Total**
$140.00	$8,649.00

Contract Period	
Option I	
Other	**Total**
$140.00	$3,652.00

Contract Period	
Option II	
Other	**Total**
$140.00	$3,652.00

Contract Period	
Option I	
Other	**Total**
$588.00	$1,884.00

Contract Period	
Option I	
Other	**Total**
$588.00	$1,884.00

Contract Period	
Option I	
Other	**Total**
$588.00	$1,884.00

MATERIALS/

Item	Manufacturer	Part Number	Unit Price	Quantity
Mealworms	Rainbow mealworms		$100/20,000	12
bat caging materials	various		$500/cage	9
bat wing bands	Porzana		$596/box	9
Cut resistant gloves	Varied		$15/pr	30
Tyvek suits	DuPOnt	EV29135313	$306/case	15
Tyvek aprons	Lakeland	6EHH7	$58/case	15
N95 respirators	3M	9511	$20/box	45
PAPRs replacement covers	3M		$96/3 units	45
cell culture flasks	Corning	430641U	415/case	5
cell culture flasks	Corning	431080	425/case	10
Nunc cell factories	Nunc	140250	$370/case	12
96 well plates	Corning	3599	$600/case	8
fetal bovine serum	GE Hyclone	SH30071.03	$600/bottle	8
DMEM medium	GE Hyclone	SH30021.02	$30/l	10
pipette tips	Fisher	13-676-10	$100/case	50
Selamectin	Zoetis		$250	
glycerin jelly	Carolina Biological Supply		$43 bottle	50
rhodamine B	Sigma		$56/100g	6
Harp Trap	Bat conservation and management		$2,003	2
hair collection bags	U-line		$75/box	10
Consumables	miscellaneous			

Note:
Consumables may be listed as a lump sum if no individual item is over $5,000. For those items that are ov

'EQUIPMENT

Total Price	Contract Period	Additional Information
1,200	Y1-Y3	
4,500	Y1-Y3	custom made
4,768	Y1-Y3	
450	Y1-Y3	
4,590	Y1-Y3	
870	Y1-Y3	
900	Y1-Y3	
4320	Y1-Y3	
2075	Y1-Y3	
4250	Y1-Y3	
4440	Y1-Y1-Y3	
4800	Y1-3.5	
4800	Y1-Y3	
300	Y1-Y3	
5000	Y1-Y3.5	
250	Y1-Y3	
2150	Y1-Y3	
336	Y1-Y3	
4006	Y1	
750	Y1-Y3	
5,344	Y1-Y3.5	needles, syringes,whirl paks, plastic bags, other disposables, all <5K
60,099		

er $5,000, list separately from the rest of consumable pricing.

60099

OTHER DI

Year	Description	Total Price	Contract Period
1	animal perdiem costs	$12,600	Y1
2	animal perdiem costs	$12,600	Y2
3	animal perdiem costs	$12,600	Y3
	rabies prphylactic shots	$4,020	Y1
	rabies prphylactic shots	$4,020	Y1
	rabies prphylactic shots	$4,020	Y1
		$49,860	

IRECT COSTS

Additional Information
up to 60 bats for 120 days at $105/day in BSL3 animal facility, includes daily husbandry, gut-loading meal worms, cleaning cages, feeding bats, veterinary services and daily surcharge for rom use,
up to 60 bats for 120 days at $105/day in BSL3 animal facility (ame as above)
up to 60 bats for 120 days at $105/day in BSL3 animal facility (same as above)
all animal care and technical staff must be vaccinated against rabies to work with bats. 1005/person
all animal care and technical staff must be vaccinated against rabies to work with bats. 1005/person
all animal care and technical staff must be vaccinated against rabies to work with bats. 1005/person

Fwd: Support letter (PREEMPT)

Rocke, Tonie E <trocke@usgs.gov>

Tue 3/20/2018 12:54 PM

To: Sleeman, Jonathan M <jsleeman@usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>

Finally, EHA sent this letter they need signed, but I have no idea how to address this issue of eligibility. I have asked for more information and perhaps for a name at DARPA we can call. Perhaps you could at least edit the letter as you see fit or perhaps you know what to do about this eligibility question. Thanks -Tonie

---------- Forwarded message ----------
From: **Luke Hamel** <hamel@ecohealthalliance.org>
Date: Tue, Mar 20, 2018 at 12:26 PM
Subject: Support letter (PREEMPT)
To: "Rocke, Tonie" <trocke@usgs.gov>
Cc: Rachel Abbott <rabbott@usgs.gov>, Jonathon Musser <musser@ecohealthalliance.org>, Evelyn Luciano <luciano@ecohealthalliance.org>

Hi Tonie,

Please see the required **collaborator support letter (attached) and comments within.** Thank you for your patience and please let me know if you have any questions.

Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

--
Tonie E. Rocke
USGS National Wildlife Health Center

6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

DARPA proposal budget to review

Rocke, Tonie E <trocke@usgs.gov>

Mon 3/19/2018 8:54 PM

To: Meicher, Lisa K <lmeicher@usgs.gov>; Richgels, Katherine L <krichgels@usgs.gov>

Hi Lisa: Please review the attached budget to make sure it is correct. Katie and I have talked about it and agreed to the categories. Katie, I reduced my salary to 0.6 month/year (5% of my time) b/c I couldn't fit in the travel budget otherwise. Let me know if you have questions. If any changes need to be made, I'll adjust supplies to keep the same total budget. Thanks -Tonie

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

WORKSPACE FORM

1-800-518-4726
SUPPORT@GRANTS.GOV

This Workspace form is one of the forms you need to complete prior to submitting your Application Package. This form can be completed in its entirety offline using Adobe Reader. You can save your form by clicking the "Save" button and see any errors by clicking the “Check For Errors” button. In-progress and completed forms can be uploaded at any time to Grants.gov using the Workspace feature.

When you open a form, required fields are highlighted in yellow with a red border. Optional fields and completed fields are displayed in white. If you enter invalid or incomplete information in a field, you will receive an error message. Additional instructions and FAQs about the Application Package can be found in the Grants.gov Applicants tab.

OPPORTUNITY & PACKAGE DETAILS:

Opportunity Number:	HR001118S0017
Opportunity Title:	PREventing EMerging Pathogenic Threats
Opportunity Package ID:	PKG00237724
CFDA Number:	12.910
CFDA Description:	Research and Technology Development
Competition ID:	
Competition Title:	
Opening Date:	01/19/2018
Closing Date:	03/27/2018
Agency:	DARPA - Biological Technologies Office
Contact Information:	BAA Coordinator PREEMPT@darpa.mil

APPLICANT & WORKSPACE DETAILS:

Workspace ID:	WS00094394
Application Filing Name:	Project DEFUSE
DUNS:	0770900660000
Organization:	ECOHEALTH ALLIANCE INC.
Form Name:	R & R Subaward Budget 10 YR Subform
Form Version:	1.4
Subform Name:	USGS Ntl. Wildlife Health Cen
Requirement:	Optional
Download Date/Time:	Mar 06, 2018 05:28:38 PM EST
Form State:	Error(s)

FORM ACTIONS:

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 1** **Start Date:** 10/01/2018 **End Date:** 09/30/2019

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Tonie		Rocke		129,590.00	0.60			6,479.00	1,747.00	8,226.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Coordinates animal studies										

Additional Senior Key Persons: [Add Attachment] [Delete Attachment] [View Attachment]

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 85,202.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00		24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel: 24,782.00

Total Salary, Wages and Fringe Benefits (A+B): 109,984.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	4,905.00
2. Foreign Travel Costs	3,614.00
Total Travel Cost	8,519.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	23,568.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.		
10.		
	Total Other Direct Costs	36,168.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	154,671.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	154,671.00	99,829.00

Total Indirect Costs 99,829.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number) USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	254,500.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	254,500.00

L. Budget Justification

(Only attach one file.) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 2** **Start Date:** 10/01/2019 **End Date:** 09/30/2020

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Tonie		Rocke		129,590.00	0.60			6,479.00	1,747.00	8,226.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Coordinates animal studies										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 85,202.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00		24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel: 24,782.00

Total Salary, Wages and Fringe Benefits (A+B): 109,984.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	1,884.00
2. Foreign Travel Costs	8,649.00
Total Travel Cost	10,533.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	21,554.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.		
10.		
	Total Other Direct Costs	34,154.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	154,671.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	154,671.00	99,829.00

Total Indirect Costs 99,829.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	254,500.00

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	254,500.00

L. Budget Justification

(Only attach one file.) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 3** **Start Date:** 10/01/2020 **End Date:** 09/30/2021

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Tonie		Rocke		129,590.00	0.60			6,479.00	1,747.00	8,226.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Coordinates animal studies										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 85,202.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00		24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel: 24,782.00

Total Salary, Wages and Fringe Benefits (A+B): 109,984.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	3,700.00
2. Foreign Travel Costs	3,614.00
Total Travel Cost	7,314.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	24,773.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.		
10.		
	Total Other Direct Costs	37,373.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	154,671.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	154,671.00	99,829.00

Total Indirect Costs 99,829.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	254,500.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	254,500.00

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 4

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 4** **Start Date:** 10/01/2021 **End Date:** 04/30/2022

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	0.60			6,479.00	1,747.00	8,226.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	6.00			30,502.00	7,986.00	38,488.00
Project Role:	collate data, assist with reprots writing and publication										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 46,714.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						

Total Number Other Personnel

Total Other Personnel

Total Salary, Wages and Fringe Benefits (A+B) 46,714.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	1,896.00
2. Foreign Travel Costs	
Total Travel Cost	1,896.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	9,532.00
2.	Publication Costs	4,000.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	
9.		
10.		
	Total Other Direct Costs	13,532.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	62,142.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	62,141.00	40,108.00

Total Indirect Costs 40,108.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	102,250.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	102,250.00

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		302,320.00
Section B, Other Personnel		74,346.00
Total Number Other Personnel	9	
Total Salary, Wages and Fringe Benefits (A+B)		376,666.00
Section C, Equipment		
Section D, Travel		28,262.00
1. Domestic	12,385.00	
2. Foreign	15,877.00	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		121,227.00
1. Materials and Supplies	79,427.00	
2. Publication Costs	4,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	37,800.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		526,155.00
Section H, Indirect Costs		339,595.00
Section I, Total Direct and Indirect Costs (G + H)		865,750.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		865,750.00

Re: [Reminder] PREEMPT call, Wed. 3/21

Rocke, Tonie E <trocke@usgs.gov>

Mon 3/19/2018 1:39 PM

To: Luke Hamel <hamel@ecohealthalliance.org>

Just to be clear, I will NOT be able to sign the document on behalf of NWHC. -Tonie

On Mon, Mar 19, 2018 at 1:07 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:

> Something I will pass along to the team here at EHA. It's possible that we will have you sign the document on behalf of NWHC. Thank you for the information!
>
> Best,
>
> **Luke Hamel**
> *Program Assistant*
>
> EcoHealth Alliance
> 460 West 34th Street – 17th floor
> New York, NY 10001
>
> (b) (6) (direct)
> (b) (6) (mobile)
> www.ecohealthalliance.org
>
> ***EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.***
>
> On Mon, Mar 19, 2018 at 2:05 PM, Rocke, Tonie <trocke@usgs.gov> wrote:
>
> > Also, to be honest, I don't know how long it will take my people to sign it. -T
> >
> > On Mon, Mar 19, 2018 at 1:04 PM, Rocke, Tonie <trocke@usgs.gov> wrote:
> >
> > > great-thanks! I just didn't want it to fall through the cracks since I am leaving the country on Thursday. -Tonie
> > >
> > > On Mon, Mar 19, 2018 at 12:55 PM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
> > >
> > > > Yes, of course - my apologies for the delay. I am working with Jonathon and Evelyn to finalize the document that we will send to you. Our plan is to send it to you later this afternoon.
> > > >
> > > > Best,

Luke Hamel
Program Assistant

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Mon, Mar 19, 2018 at 1:21 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Please don't forget to send me a template or whatever is you need my director to sign off on. I need to get that done before Wednesday. -Tonie
>
> On Mon, Mar 19, 2018 at 8:27 AM, Luke Hamel <hamel@ecohealthalliance.org> wrote:
>
> > Hi Tonie,
> >
> > This is just a reminder that our next PREEMPT call will take place on **Wed. 3/21 @ 10 AM ET**.
> >
> > Phone: 1-719-785-9461
> > Password: 9784#
> >
> > Best,
> >
> > **Luke Hamel**
> > *Program Assistant*
> >
> > EcoHealth Alliance
> > 460 West 34th Street – 17th floor
> > New York, NY 10001
> >
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> > www.ecohealthalliance.org
> >
> > ***EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and***

> ***delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.***

--
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Re: DARPA PRE-EMPT

Rocke, Tonie E <trocke@usgs.gov>

Fri 3/16/2018 3:51 PM

To: Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>

Hi Jerome: Sure, here's my draft narrative. I was planning to update it once we figured out if we could contract with you and after Peter reviewed it. Also, I have no idea what to put as a start date and haven't got a response to my inquiry about it. So I guessed at 10/1/18 but I'd be surprised if it comes through that fast. -Tonie

On Fri, Mar 16, 2018 at 3:41 PM, <Jerome.Unidad@parc.com> wrote:

> Tonie,
>
> Thanks for your email. Just for organizational purposes so we clearly know who is the technical lead for this main part. Would you be able to share the concept paper that you submitted to DARPA prior to this full proposal? Just for our information. If not, I will just wait for Peter to send the full integrated proposal and identify the blanks we might need to fill in.
>
> We really appreciate your effort in bringing us in. Our team is very excited to be involved in this project.
>
> Best,
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company
>
> **From:** Rocke, Tonie [mailto:trocke@usgs.gov]
> **Sent:** Friday, March 16, 2018 12:39 PM
> **To:** Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
> **Subject:** Fwd: DARPA PRE-EMPT
>
> Hi Jerome: I think we'll be your point of contact for most of the project, and we will probably use both biomarker analysis and viral load in fecal samples as response indicators for the final field trial where several investigators will be involved. I asked EHA to provide the subcontract to you

instead of us to avoid possible hassles with it needing to go out for bid from our contracting office. Thanks for being flexible! -Tonie

---------- Forwarded message ----------
From: **Anna Willoughby** <willoughby@ecohealthalliance.org>
Date: Fri, Mar 16, 2018 at 2:12 PM
Subject: Re: DARPA PRE-EMPT
To: Jerome.Unidad@parc.com
Cc: "William B. Karesh" <karesh@ecohealthalliance.org>, Peter Daszak <daszak@ecohealthalliance.org>, trocke@usgs.gov, Luke Hamel <hamel@ecohealthalliance.org>, Alison Andre <andre@ecohealthalliance.org>, Amanda Fuchs <amanda.andre@ecohealthalliance.org>, Kateri.Paul@parc.com

Thanks for these details, Jerome. Attached are my notes from the call. Action items include:

- Jerome to send more detailed scope of work with paragraphs and revised budget by early next week

- EHA will send PARC the NWHC section of the proposal on Monday
- EHA will send the format of letter of support for PARC
- EHA to follow up with Kateri with requested information

For your question on collaborating with other institutes, it is likely that all organizations involved may have insight into the aerosol-bat interaction. I believe this topic would be covered during the Annual Meeting between all partners, as well as during relevant cross-partner trips, in addition to monthly conference calls.

Please let us know if you have further questions.

Best,
Anna

On Fri, Mar 16, 2018 at 2:57 PM, <Jerome.Unidad@parc.com> wrote:

> An additional point for Peter, Tonie (and everyone),
>
> For the spray technology, refinement of the details with respect to aerosol-bat interaction (i.e. the preliminary field testing to see how bats react to the aerosol) and eventual field-deployment in China, will the technical lead for coordinating this segment of the project be USGS – National Wildlife Center? Or should we also expect to work/coordinate with other institutes who would give feedback and insights on how this works?
>
> Thanks. This is just for our information.

Best,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Unidad, Jerome <Jerome.Unidad@parc.com>
Sent: Friday, March 16, 2018 11:52 AM
To: 'William B. Karesh' <karesh@ecohealthalliance.org>; 'Peter Daszak' <daszak@ecohealthalliance.org>
Cc: 'Luke Hamel' <hamel@ecohealthalliance.org>; 'Anna Willoughby' <willoughby@ecohealthalliance.org>; 'Alison Andre' <andre@ecohealthalliance.org>; 'Amanda Andre' <amanda.andre@ecohealthalliance.org>; 'Rocke, Tonie' <trocke@usgs.gov>; Paul, Kateri <Kateri.Paul@parc.com> <Kateri.Paul@parc.com>
Subject: RE: DARPA PRE-EMPT

Peter and team,

I'm currently working on putting together a revised budget and equivalent statement of work (tasks breakdown) for PARC's involvement with the project. You can expect this about early next week – approximately Monday. Officially, for the submission, our capture manager, Kateri Paul, who takes care of the other things would need the following things from your equivalent to facilitate our parts of the submission.

1. Request for Proposal that we can respond to with what they need for their package to DARPA
2. Start date of the proposed effort
3. Contract or a Grant/Other Transaction

Once we have finalized the scope of work and the budget, Kateri will be in touch for these other aspects. Her contact information can be found below.

Kateri E. Paul

Capture Manager, Public Sector

Global Business Development

Palo Alto Research Center (PARC)

3333 Coyote Hill Road

Palo Alto, CA 94304

Kateri.Paul@parc.com

650-812-4821 (desk)

617-596-2023 (mobile)

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Unidad, Jerome <Jerome.Unidad@parc.com>
Sent: Thursday, March 15, 2018 3:33 PM
To: 'Rocke, Tonie' <trocke@usgs.gov>; William B. Karesh <karesh@ecohealthalliance.org>; Johnson, David <David.Johnson@parc.com> <David.Johnson@parc.com>
Cc: Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: RE: DARPA PRE-EMPT

Dear all,

10AM-11AM PST (12PM-1PM CT, 1PM-2PM ET) should work for us. I shall setup a WebEx meeting for this, given the number of participants.

Let me know if this timeslot will work.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 2:39 PM
To: William B. Karesh <karesh@ecohealthalliance.org>
Cc: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I assume that is ET? -T

On Thu, Mar 15, 2018 at 4:14 PM, William B. Karesh <karesh@ecohealthalliance.org> wrote:

> Tonie and Jerome,
>
> We would still like to speak. Anytime on Friday between 11:00 AM and 2:00 PM would be great.
>
> BK
>
> **William B. Karesh, D.V.M**
>
> *Executive Vice President for Health and Policy*
>
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President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

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On Mar 15, 2018, at 4:55 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

Hi all: Since we didn't hear back from EcoHealth Alliance, Jerome and I went ahead with a short call we had been planning anyway regarding some technical details. I told him our concerns about the proposed budget and we think we have a pretty good plan to reduce the scope of work to the funds we have available. PARC is very unique in developing this technology and their technology fits very well with other work I am doing, so we both feel pretty confident we can work something out. If you still wish to have a discussion among all of us, we can schedule that for tomorrow, as I believe Jerome had another meeting to run off to for the rest of the day. I'm available the rest of the day if you wish to chat about this in person. Best -Tonie

On Thu, Mar 15, 2018 at 3:42 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

> Actually – can we do a phone call – I'll be driving. 5.15pm would be perfect (NYC time), Today Thursday.

Is that possible?

Our call in line is: 1-719-785-9461

Passcode: 9784#

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

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@PeterDaszak

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Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: William B. Karesh [mailto:karesh@ecohealthalliance.org]
Sent: Thursday, March 15, 2018 12:49 PM
To: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
Cc: Rocke, Tonie <trocke@usgs.gov>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
Subject: DARPA PRE-EMPT

Dear Dr. Unidad,

Thanks for your quick responses to Dr. Rocke. Would you be available for a short call with Dr. Daszak, Dr. Rocke and me this afternoon or Friday.

We're on tight timeline so we thought a phone call might be save quite a bit of time.

Thanks in advance,

Billy

William B. Karesh, D.V.M

Executive Vice President for Health and Policy

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--

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--

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Task 7: Develop and assess delivery methods to bats for immune boosting and priming molecules

Description and execution: While work is proceeding to identify and optimize immunomodulating agents to manage SARS-Coronaviruses, we will concurrently develop and test mediums, routes, and methods of delivery to large colonies of bats. Several different approaches or combinations of approaches will be assessed to determine the most feasible and simplest method of delivery that achieves high uptake by bats, is safe for humans as well as target and non-target species, and minimizes disturbance to the colony. Sticky edible gels or pastes that bats groom from themselves and each other have been used previously to deliver pharmaceuticals to bats orally and are currently being tested as a medium for delivery of vaccines against rabies and other diseases in wild bats (see preliminary data). These may also be useful for delivering immune modulators and recombinant SARSr-CoV spike proteins to *Rhinolophus* bats, but may need to be combined with viral vectors (like poxvirus or adenovirus) or nanoparticles/nanoemulsions that enhance uptake through mucous membranes or transdermally after topical application.

Poxviruses in particular have been demonstrated to be effective viral vectors for delivering vaccines to wildlife (Slate et al., 2009) Freuling et al., 2013; Rocke et al., 2017). Recent laboratory studies in bats have shown that poxviruses can replicate safely at high levels in bats after oronasal administration (Stading et al., 2016)m and poxvirus vectored vaccines are immunogenic, protecting bats from rabies challenge (Stading et al 2017; see preliminary data). Poxviruses are highly safe, having been tested in a wide variety of wild and domestic animals, they allow for large inserts of foreign DNA, and they have a proven record of success. Poxviruses are good candidates for this project, but we will also consider others.

In addition to viral vectors, we will also consider methods to achieve transcutaneous delivery of the immune boosting proteins without the use of live agents. Recent advances in methods to achieve transdermal or transcutaneous delivery of drugs and vaccines have been reported. (Roberts et al., 2017). However, a major impediment to this route of vaccination is the stratum corneum, the outermost barrier layer of the skin that protects underlying layers from infection and damage. Numerous approaches have relied on mechanical methods to compromise the stratum corneum to allow the drug or vaccine to penetrate into the skin (Roberts et al., 2017). Innovations in nanotechnology show promise in being able to deliver drugs and vaccines into the deeper layers of the skin without the need for damage to the stratum corneum (Mishra et al., 2013), an important consideration. Dendritic cells and Langerhans cells, antigen-presenting cells which reside in the dermis and epidermis, can take up these transdermally delivered proteins and generate an immune response. We are currently testing poly lactic-co-glycolic acid (PLGA) as a nanoparticle to encapsulate rabies glycoprotein as a method of transcutaneous delivery of vaccine to bats. PLGA has been used previously to deliver both toll-like receptor agonists and antigens simultaneously to mice (Ebrahimian, 2017). This and other products (outlined above in Task ?) could potentially be useful with SARSr-CoV glycoproteins. Adjuvants can also be incorporated into nanoemulsions and nanoparticles to amplify the natural immune response to the vaccine antigens (Karande and Mitragotri, 2010). With SARS-CoV spike proteins, the adjuvant Matrix M1

(Isconova, Sweden) has been shown to significantly enhance the immune response in mice (Coleman et al. 2014)

In collaboration with Dr. Baric and others, we will determine the most likely immunomodulating formulations based on the results of TA2, previous animal studies and other available data and then use both laboratory and field studies to assess and optimize delivery vehicles and methods for wild bats. To reduce costs, initial studies will be conducted with locally acquired insectivorous bats (*Eptesicus fuscus*--big brown bats). We have successfully maintained and housed big brown bats and other insectivorous species for several experiments at our facility previously (Stading et al., 2016, 2017). We will treat bats via topical application with various test formulations that include the biomarker Rhodamine B (RB), co-house them with untreated bats, and monitor transfer between bats by collecting hair and whiskers for biomarker analysis. Rhodamine B is detectable within the hair of animals within 24 hours of consumption using a fluorescence microscope, and we have considerable experience using this biomarker for similar studies (see preliminary data).

Once we have confirmed uptake in laboratory studies, we will then assess mass delivery methods in local caves and hibernacula (using biomarker-labeled mediums but without immunomodulatory substances). We will test several different approaches including aerosolization via sprayers that could be used in cave settings and automated sprays triggered by timers and movement detectors at critical cave entry points. Within one week of application, bats will be trapped at the cave entrace using mist nets or Harp traps and hair will be collected to assess the rate of uptake via biomarker analysis. The bats will be released immediately afterward. The procedures will be tested at several different locations as it will likely take some manipulation to determine appropriate dosages for maximum uptake. After we have determined the most optimal approaches for mass delivery, we will then test them on wild bats in our three cave sites in Yunnan Province. Again, biomarker will be used to assess rates of uptake and this data can then be used in modeling studies to help determine the optimal rates of application of immunomodulating agents. Biomarker studies can also be used to assess uptake by non-target species, an important consideration in evaluating safety. Fieldwork will be conducted in collaboration with Dr. Yunzhi Zhang (Yunnan CDC, Consultant at EcoHealth Alliance).

Preliminary Data: Rocke and colleagues have developed oral vaccines and delivery methods to manage disease in free-ranging wildlife for many years, including a sylvatic plague vaccine for prairie dogs (Rocke et al., 2017), and more recently, vaccines against rabies (Stading et al., 2017) and white-nose syndrome for bats (Rocke, unpublished data). In addition to developing, testing and registering vaccines for experimental field use, vaccine delivery methods and uptake by the target species were optimized using biomarker studies prior to deployment; biomarker studies were also used to assess uptake and safety in non-target hosts (Tripp et al., 2015). A similar approach will be used to develop, test and optimize delivery methods to *Rhinolophus* bats in SE Asia.

To manage plague caused by *Yersinia pestis* in prairie dogs, a raccoon poxvirus vectored vaccine expressing plague antigens was incorporated into a peanut-butter flavored bait matrix. Rhodamine B (RB), a biomarker that dyes hair, whiskers and feces and is visible within 24 hours of consumption by animals, was included in the baits in

order to assess uptake by both target and non-target species (Figure 1). When viewed under a UV microscope at a specific wavelength, the biomarker is visible until the hair grows out (approximately 50 days in prairie dogs). Biomarker studies were initially used to assess palatability and acceptance of the bait matrix by wild prairie dogs (Tripp et al., 2014) and also used to assess bait ingestion by non-target rodents (Tripp et al., 2015). After safety was confirmed in non-targets and with the approval of USDA Center for Veterinary Biologics, a large field trial was conducted over a 3-year period that demonstrated vaccine effectiveness in four species of prairie dogs in seven western states (Rocke et al., 2017). Using biomarker analysis, we then assessed site- and individual host-level factors related to bait consumption in prairie dogs to determine those most related to increased bait consumption, including age, weight, and the availability of green vegetation. Identifying the factors that maximize the likelihood of expedient bait uptake by targeted individuals is important for developing strategies to optimize vaccine effectiveness. This will also be important in developing disease management strategies for bats.

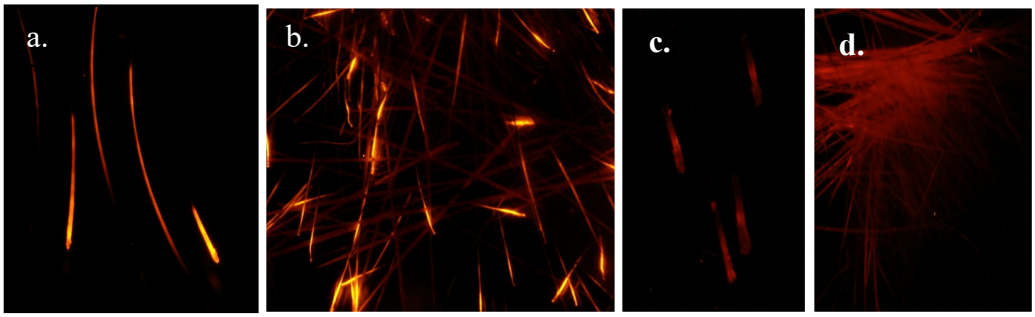

Figure 1. Prairie dog hair and whisker samples viewed under fluorescence microscope (excitation wavelength: 540 nm, emission wavelength: 625 nm) to determine uptake of baits containing Rhodamine B. a) whiskers positive for RB uptake 20 days after bait distribution, b) hair sample positive for RB uptake 16 days after bait distribution, c and d) whiskers and hair negative for RB uptake 20 days after bait distribution (note natural dull fluorescence).

In recent years, our research team has been developing and testing vaccines and delivery methods for use in free-ranging bats. First we tested two commonly used viral vectors, modified vaccinia Ankara (MVA) and raccoon poxvirus (RCN), for their safety and replication in bats using in vivo biophotonic imaging. (Stading et al. 2017). RCN replicated to higher levels in bats than MVA, even via the oral route, and was found to be highly safe for bats (Figure 2). We then used raccoon poxvirus as a viral vector to express a novel rabies glycoprotein (mosaic or MoG) and tested the protective efficacy of this construct in bats after both oronasal and topical administration (Stading et al 2017). Both methods of application were successful, protecting nearly all of the immunized and challenged bats (Figure 3), work is now progressing to develop methods of vaccine delivery to vampire bats, one of the primary reservoirs of rabies for both humans and animals, primarily cattle, in several Latin American countries. We are also using a similar approach to develop vaccines for white-nose syndrome in bats, a devastating disease that has killed millions of insectivorous bats in North America.

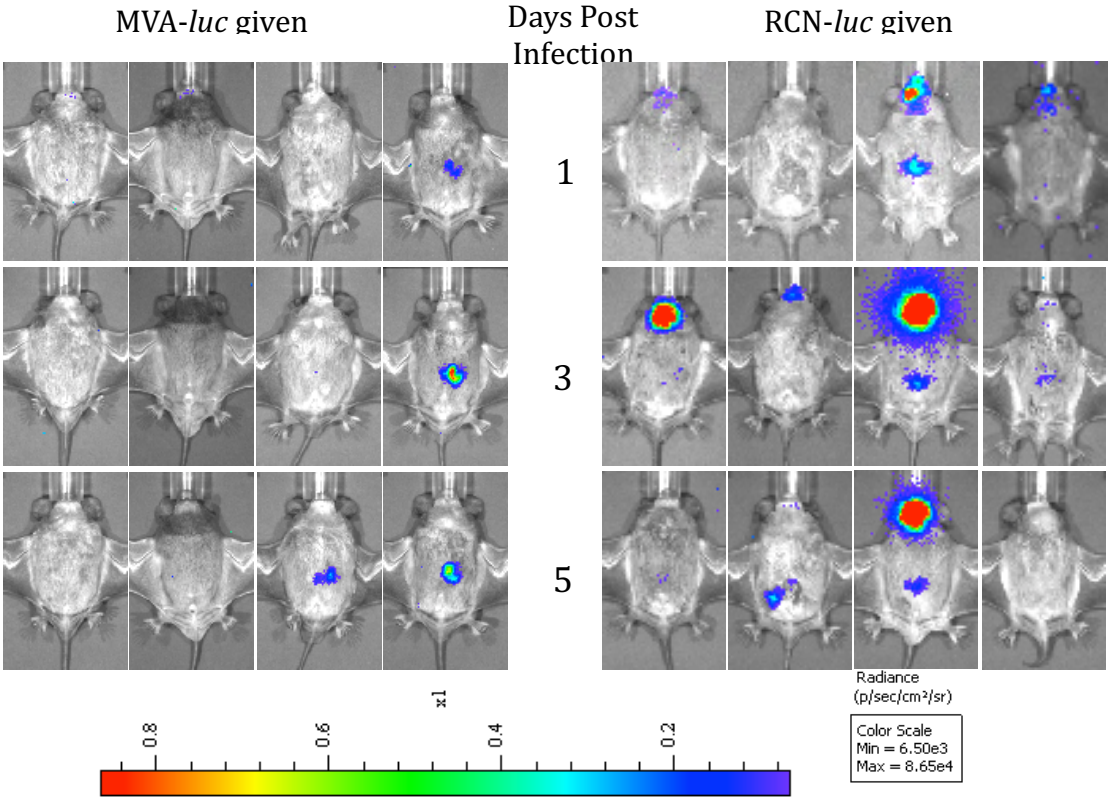

Figure 2. Luminescence, indicative of viral replication of modified vaccinia Ankara (MVA) and raccoon poxvirus RCN) in *Tadarida brasiliensis* on days 1, 3 and 5 post-inoculation via the oronasal route.

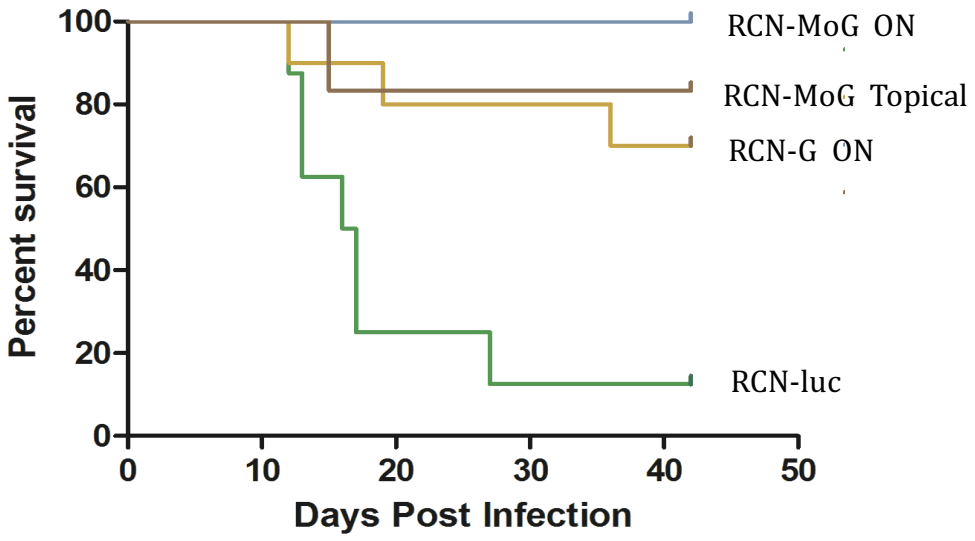

Figure 3. Results of vaccine efficacy and rabies challenge trials in *Epstesicus fuscus* immunized with raccoon poxvirus expressing a mosaic G protein (RCN-MoG) either oronasally (ON) or topically in comparison to RCN expressing typical G protein and RCN expressing luciferase (a negative control).

For bats a different approach is required for vaccine delivery, as in general, they are not attracted to baits. Bats, especially vampire bats, are known to practice self and mutual grooming at a high rate, and this behavior has been exploited to cull vampire bats using poisons like warfarin. The poison is applied topically to a number of bats that are released. When they return to their roost, the poison is transferred to roost-mates by contact and mutual grooming. We are exploiting this same behavior for vaccine application. Preliminary biomarker studies (without vaccine) are being conducted in vampire bats in both Mexico and Peru and also in insectivorous bats in Wisconsin. In a pilot study in Peru, we treated 50 bats from a single cave with RB-labelled glycerin jelly. Based on capture-recapture data, we estimated the population at ~200 bats, so ~25% of bats were initially marked. Upon trapping of this population a few days later, 64 bats were captured, including 19 originally marked bats (Table 1 – could be made into a figure instead). Hair was collected and examined for RB marking under a fluorescence microscope. All treated bats were positive for RB marking in addition to 39% of newly captured bats, indicating a rate of transfer of about 1.3 bats for every bat marked. Additional trials have been conducted, with transfer rates of up to 2.8 bats for every bat treated achieved at least once. These trials are being analyzed to assess factors associated with rates of transfer, e.g. sex and age of initially treated bats, time of day, etc. This data is then being used to model the rate of vaccination and impact on rabies transmission with different rates of application, prior to actual deployment of vaccine in the field.

Table 1. Marking of vampire bats a few days after application of glycerin jelly containing Rhodamine B.

	Number captured	**Positive**	**Negative**	**Inconclusive**	**% positive (w/o inc)**
All bats	64	34	25	5	58
Recaptured marked bats	19	18	0	1	100
New bat captures	45	16	25	4	39

For insectivorous bats, we are trying other approaches. Instead of hand applying the jelly to bats, we applied RB marked glycerin jelly to the entry of bat houses used by little brown bats (*Myotis lucifugus*). The bats became covered as they entered the houses and then consumed the material during self and mutual grooming. One week later, bats were trapped at the houses to determine the rate of uptake. Of 29 bats trapped one week post-application, 59% (17) were positive for biomarker indicating they had eaten the jelly. Thus, with additional optimization, application of vaccine to bat houses or other

structures (small cave entrances) could also be a viable method of delivery. In addition, we are considering different spray applications directly to roosting bats in caves and through motion-sensing sprayers at cave entrances. Whatever the means of application, effective treatment relies on ingestion by bats, and that is easily confirmed with the use of the biomarker, RB.

Organization leading task: USGS National Wildlife Health Center

Progress Metrics: Not sure exactly what format to use here

Deliverable(s):
Medium and methods to deliver immunomodulatory agents to bats.
Data on uptake in insectivorous bats.
Reports, manuscripts, presentations.

Coleman CM, Liu YV, Mu H, Taylor JK, Massare M, Flyer DC, Smith GE, Frieman MB. 2014. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine 32:3169-3174.

Ebrahimian M, Hashemi M, Maleki M, Hashemitabar G, Abnous K, Ramezani M, Haghparast A. 2017. Co-delivery of dual toll-like receptor agaonists and antigen in poly(lactic-co-glycolic) acid/polyethylenimine cationic hybrid nanoparticles promote efficient in vivo immune responses. Front Immunol 8:1077.

Freuling CM, Hampson K, Selhorst T, Schro¨der R, Meslin FX, Mettenleiter TC, Mu¨ller T (2013) The elimination of fox rabies from Europe: determinants of success and lessons for the future. Philosophical Transactions of the Royal Society London B Biological Sciences 368(1623):20120142 (DOI: 10.1098/rstb.2012. 0142)

Karande P, Mitragotri S. 2010. Transcutaneous immunization: an overview of advantages, disease targets, vaccines, and delivery technologies. Annu Rev Chem Biomol Eng 1:175-201.

Mishra DK, Dhote V, Mishra PK. 2013. Transdermal immunization: biological framework and translational perspectives. Expert Opin Drug Deliv 10:183-200.

Roberts MS, Mohammed Y, Pastore MN, Namjoshi S, Yousef S, Alinaghi A, Haridass IN, Abd E, Leite-Silva VR, Benson HAE, Grice JE. 2017. Topical and cutaneous delivery using nanosystems. J Control Release 247:86-105.

Rocke TE, Tripp DW, Russell RE, Abbott RC, Richgels KLD, Matchett MR, Biggins DE, Griebel R, Schroeder G, Grassel SM, Pipkin DR, Cordova J, Kavalunas A, Maxfield B, Boulerice J, Miller MW. 2017. Sylvatic plague vaccine partially protects prairie dogs (*Cynomys* spp.) in field trials. EcoHealth DOI: 10.1007/s10393-017-1253-x.

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Stading BR, Osorio JE, Velasco-Villa A, Smotherman M, Kingstad-Bakke B, Rocke TE. Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). Vaccine. 2016;34: 5352–5358. doi:10.1016/j.vaccine.2016.08.088

Stading B, Ellison JA, Carson WC, Panayampalli SS, Rocke TE, Osorio JE. Protection of bats (*Eptesicus fuscus*) against rabies following topical or oronasal exporue to a recombinant raccoon poxvirus vaccine. PLoS Negl Trop Dis 11:e0005958.

Tripp DW, Rocke TE, Streich SP, Brown NL, Fernandez JR-R, Miller MW. 2014. Season and application rates affect vaccine bait consumption by prairie dogs in Colorado and Utah, USA. J Wildlife Dis 20:

Tripp DW, Rocke TE, Streich SP, Abbott RC, Osorio JE, Miller MW. 2015. Apparent field safety of a raccoon poxvirus-vectored plague vaccine in free-ranging prairie dogs, Colorado, USA. J Wildlife Dis 51:

Fwd: DARPA PRE-EMPT

Rocke, Tonie E <trocke@usgs.gov>

Fri 3/16/2018 2:38 PM

To: Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>

Hi Jerome: I think we'll be your point of contact for most of the project, and we will probably use both biomarker analysis and viral load in fecal samples as response indicators for the final field trial where several investigators will be involved. I asked EHA to provide the subcontract to you instead of us to avoid possible hassles with it needing to go out for bid from our contracting office. Thanks for being flexible! -Tonie

---------- Forwarded message ----------
From: **Anna Willoughby** <willoughby@ecohealthalliance.org>
Date: Fri, Mar 16, 2018 at 2:12 PM
Subject: Re: DARPA PRE-EMPT
To: Jerome.Unidad@parc.com
Cc: "William B. Karesh" <karesh@ecohealthalliance.org>, Peter Daszak <daszak@ecohealthalliance.org>, trocke@usgs.gov, Luke Hamel <hamel@ecohealthalliance.org>, Alison Andre <andre@ecohealthalliance.org>, Amanda Fuchs <amanda.andre@ecohealthalliance.org>, Kateri.Paul@parc.com

Thanks for these details, Jerome. Attached are my notes from the call. Action items include:

- Jerome to send more detailed scope of work with paragraphs and revised budget by early next week
- EHA will send PARC the NWHC section of the proposal on Monday
- EHA will send the format of letter of support for PARC
- EHA to follow up with Kateri with requested information

For your question on collaborating with other institutes, it is likely that all organizations involved may have insight into the aerosol-bat interaction. I believe this topic would be covered during the Annual Meeting between all partners, as well as during relevant cross-partner trips, in addition to monthly conference calls.

Please let us know if you have further questions.

Best,
Anna

On Fri, Mar 16, 2018 at 2:57 PM, <Jerome.Unidad@parc.com> wrote:

> An additional point for Peter, Tonie (and everyone),
>
> For the spray technology, refinement of the details with respect to aerosol-bat interaction (i.e. the preliminary field testing to see how bats react to the aerosol) and eventual field-deployment in China, will the technical lead for coordinating this segment of the project be USGS – National Wildlife Center? Or should we also expect to work/coordinate with other institutes who would give feedback and insights on how this works?
>
> Thanks. This is just for our information.
>
> Best,
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company
>
> ---
>
> **From:** Unidad, Jerome <Jerome.Unidad@parc.com>
> **Sent:** Friday, March 16, 2018 11:52 AM
> **To:** 'William B. Karesh' <karesh@ecohealthalliance.org>; 'Peter Daszak' <daszak@ecohealthalliance.org>
> **Cc:** 'Luke Hamel' <hamel@ecohealthalliance.org>; 'Anna Willoughby' <willoughby@ecohealthalliance.org>; 'Alison Andre' <andre@ecohealthalliance.org>; 'Amanda Andre' <amanda.andre@ecohealthalliance.org>; 'Rocke, Tonie' <trocke@usgs.gov>; Paul, Kateri <Kateri.Paul@parc.com> <Kateri.Paul@parc.com>
> **Subject:** RE: DARPA PRE-EMPT
>
> Peter and team,
>
> I'm currently working on putting together a revised budget and equivalent statement of work (tasks breakdown) for PARC's involvement with the project. You can expect this about early next week – approximately Monday. Officially, for the submission, our capture manager, Kateri Paul, who takes care of the other things would need the following things from your equivalent to facilitate our parts of the submission.
>
> 1. Request for Proposal that we can respond to with what they need for their package to DARPA

2. Start date of the proposed effort
3. Contract or a Grant/Other Transaction

Once we have finalized the scope of work and the budget, Kateri will be in touch for these other aspects. Her contact information can be found below.

Kateri E. Paul

Capture Manager, Public Sector

Global Business Development

Palo Alto Research Center (PARC)

3333 Coyote Hill Road

Palo Alto, CA 94304

Kateri.Paul@parc.com

650-812-4821 (desk)

617-596-2023 (mobile)

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Unidad, Jerome <Jerome.Unidad@parc.com>
Sent: Thursday, March 15, 2018 3:33 PM
To: 'Rocke, Tonie' <trocke@usgs.gov>; William B. Karesh <karesh@ecohealthalliance.org>; Johnson, David <David.Johnson@parc.com> <David.Johnson@parc.com>
Cc: Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: RE: DARPA PRE-EMPT

Dear all,

10AM-11AM PST (12PM-1PM CT, 1PM-2PM ET) should work for us. I shall setup a WebEx meeting for this, given

the number of participants.

Let me know if this timeslot will work.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 2:39 PM
To: William B. Karesh <karesh@ecohealthalliance.org>
Cc: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Amanda Andre <amanda.andre@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I assume that is ET? -T

On Thu, Mar 15, 2018 at 4:14 PM, William B. Karesh <karesh@ecohealthalliance.org> wrote:

> Tonie and Jerome,
>
> We would still like to speak. Anytime on Friday between 11:00 AM and 2:00 PM would be great.
>
> BK
>
> **William B. Karesh, D.V.M**

Executive Vice President for Health and Policy

EcoHealth Alliance

460 West 34th Street - 17th Floor

New York, NY 10001 USA

+1.212.380.4463 (direct)

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President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

> On Mar 15, 2018, at 4:55 PM, Rocke, Tonie <trocke@usgs.gov> wrote:
>
> Hi all: Since we didn't hear back from EcoHealth Alliance, Jerome and I went ahead with a short call we had been planning anyway regarding some technical details. I told him our concerns about the proposed budget and we think we have a pretty good plan to reduce the scope of work to the funds we have available. PARC is very unique in developing this technology and their technology fits very well with other work I am doing, so we both feel pretty confident we can work something out. If you still wish to have a discussion among all of us, we can schedule that for

tomorrow, as I believe Jerome had another meeting to run off to for the rest of the day. I'm available the rest of the day if you wish to chat about this in person. Best -Tonie

On Thu, Mar 15, 2018 at 3:42 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

> Actually – can we do a phone call – I'll be driving. 5.15pm would be perfect (NYC time), Today Thursday.
>
> Is that possible?
>
> Our call in line is: 1-719-785-9461
>
> Passcode: 9784#
>
> Cheers,
>
> Peter
>
> **Peter Daszak**
>
> *President*
>
> EcoHealth Alliance
>
> 460 West 34th Street – 17th Floor
>
> New York, NY 10001

Tel. +1 212-380-4474

www.ecohealthalliance.org

@PeterDaszak

@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Jerome.Unidad@parc.com [mailto:Jerome.Unidad@parc.com]
Sent: Thursday, March 15, 2018 4:23 PM
To: trocke@usgs.gov
Cc: William B. Karesh; Peter Daszak; Luke Hamel
Subject: RE: DARPA PRE-EMPT

I can setup a WebEx quickly if we will have multiple parties.

Thanks,

Jerome

Jerome Unidad, PhD

Advanced Manufacturing and Deposition Systems
Hardware Systems Laboratory

PARC, A Xerox Company

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Thursday, March 15, 2018 1:22 PM
To: Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Daszak Peter <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
Subject: Re: DARPA PRE-EMPT

I'm available as well. Billy, do you have a call in number? -Tonie

On Thu, Mar 15, 2018 at 3:20 PM, <Jerome.Unidad@parc.com> wrote:

> Dear all,
>
> Sorry for the late response – yes, I will be available for a phone call now. Up to 2PM.
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company
>
> ---
>
> **From:** William B. Karesh [mailto:karesh@ecohealthalliance.org]
> **Sent:** Thursday, March 15, 2018 12:49 PM
> **To:** Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
> **Cc:** Rocke, Tonie <trocke@usgs.gov>; Peter Daszak <daszak@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>
> **Subject:** DARPA PRE-EMPT
>
> Dear Dr. Unidad,
>
> Thanks for your quick responses to Dr. Rocke. Would you be available for a short call with Dr. Daszak, Dr. Rocke and me this afternoon or Friday.
>
> We're on tight timeline so we thought a phone call might be save quite a bit of time.
>
> Thanks in advance,
>
> Billy

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New York, NY 10001

(b) (6) (direct)
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www.ecohealthalliance.org

Visit our blog: http://blog.ecohealthalliance.org/updates

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

March 15, 2018, 1pm EST
EHA: Billy Karesh, Peter Daszak, Anna WIlloughby
NWHC: Tonie Rocke
PARC: Jerome Unidad and David Johnson

Project DEFUSE, PI Peter Daszak

Budget

- Current budget is 580k for all tasks in original scope (360k development; 220 field prototype: 3-4 copies)
- EHA: Budget is currently too expensive. Avenues for reduction:
    - Reduce scope/trim intermediate steps? (not preferable)
- Original estimate is lean for prototype for scale: 2-3 bats at a time, generating aerosol for significant amount of space
- PARC may be able to make less expensive (for beginning scope of work)
- DARPA may have further, add-on support after program has begun
- Include some travel: PARC will need a cross-partner visit with EHA (or vice versa), will attend annual meeting, Y2 China visit, visit to TR captive colony in Y1.

Collaboration

- Exclusive partnership between EHA and PARC for DARPA application

Scope of Work (EHA needs more details, paragraph per item)

- Goals for EHA are to have engagement from PARC for duration of project and for deployment trials
- PARC sent white paper, should insert relevant info into proposal
- PARC want time to optimize correctly, (eg spray quality, fluid consistency)
- For DARPA purposes: proof of concept that you can interfere and disrupt v. transmission. Large scale intervention not necessary.
- Y1: Creating initial product, modifying existing fixtures; deploy biomarker in captive species, each captive bat experiment is ~32k (TR)
- Y2: Refining prototype for field use, deploy biomarker in field species stateside (TR)
    - Bats will be sampled for biomarker spread
- **EHA add link to video in proposal**

Deployment Details

- For Chinese bat caves: we would go to minor entrances/side pocket. (smaller scale, could then be scaled up after the project)
- PARC: How big are caves? EHA: Volume 2 ft by 2 ft, similar to furniture size, Not going to cave with 10,000 bats. This is simply a field trial.
- EHA: Deploy for 2-3 days at one site for field trial in China. Have at least 2 prototypes
- EHA: Will not manufacture large-scale spray material as too expensive
- Deploy Biomarker Study: Captive Bats (NWHC) -> Field (US) -> Field (China)
- Deploy Mesocosm Study: Captive Bats (Duke-NUS) -> Field (China)

-

NWHC budget

Rocke, Tonie E <trocke@usgs.gov>

Fri 3/16/2018 12:53 PM

To: Luke Hamel <hamel@ecohealthalliance.org>; Daszak Peter <daszak@ecohealthalliance.org>; Billy Karesh <(b) (6) @gmail.com>

Hi all: Here is a quick summary and my "draft" budget templates. I wanted to get this to you today so you can figure out the workflow over the weekend as you put together the proposal and so it is very clear what I can do. Note I have to get this budget approved by NWHC on Monday so something might change slightly.

You indicated you had budgeted $1,065,750 for 3.5 years for my part of the project, which primarily involves developing the appropriate delivery mechanism to bats. Based on our conversations about PARC and the need to subcontract the engineering of the spray device (which in my mind is absolutely critical to the project so glad our phone conversation with PARC went well-thanks!!) I have subtracted $200K ($50K/yr).

Bat experiments are quite costly because they require us trapping them and hand feeding on a daily basis. Also full PPE, because they potentially carry rabies (we had an outbreak in our facility last year). See below for a breakdown. So, after reducing for overhead costs, that leaves me $526,154. For that amount, this what I can give you in terms of animal experiments.

Year 1: 1 -2 experiments with up to 60 bats for up to 120 days (includes 30 day quarantine period-rabies watch) $35K (note since these are not terminal studies, we can potentially use the bats for more than 1 experiment).
Year 2: 1 -2 experiments with up to 60 bats for up to 120 days (includes 30 day quarantine period) $35K
Year 3: 1 experiment with up to 60 bats for 90 days (includes 30 day quarantine period) $26K
1 RCN construct (Ralph thought that was sufficient and any more than that would require more $). I will provide the virally vectored immunogen to Ralph and he will first conduct mouse studies, before we put it in bats.

The rest of my budget is in personnel costs, travel-estimated at this point, and a minimal supply budget.

After talking with Ralph about response variables to measure with immune boosting particles, I realized we can easily do an experiment to evaluate an RCN construct as he can measure serologic response. However, the other products (agonists, adjuvants) would require quite a bit of additional work which I can not provide, unless it is simply providing frozen tissue to another partner (not sure about that and Ralph wasn't either). That needs to be done elsewhere, and in any case these products should be tested in the target species. In addition to the RCN study, I will assess methods/mediums of delivery, dosages, application rates, etc. We need to figure out how much to apply to a bat topically to deliver the appropriate dosage for consumption. We would also work with Ralph's group to assess nanoparticle uptake, dosage, etc., and work with Parc to assess how their delivery system is working first in the lab and then in the field.

Let me know if you have questions or concerns.

Peter, I will wait to get a new draft from you before updating my section, but just a reminder, I need to provide finals to you before COB 3/21 as I won't have easy access to email in Mexico.

Thanks and good luck this weekend pulling it all together. -Tonie

Bat care costs (based on a 60-bat experiment for ~120 days in 2017)
$105/day per diem ($12,600)
720 man hours of hand feeding ($12,240)
food, PPE, drugs, rabies shots, caging materials ($10,000)
trapping ($400)
Total: $35,240K

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451

trocke@usgs.gov

WORKSPACE FORM

1-800-518-4726
SUPPORT@GRANTS.GOV

This Workspace form is one of the forms you need to complete prior to submitting your Application Package. This form can be completed in its entirety offline using Adobe Reader. You can save your form by clicking the "Save" button and see any errors by clicking the “Check For Errors” button. In-progress and completed forms can be uploaded at any time to Grants.gov using the Workspace feature.

When you open a form, required fields are highlighted in yellow with a red border. Optional fields and completed fields are displayed in white. If you enter invalid or incomplete information in a field, you will receive an error message. Additional instructions and FAQs about the Application Package can be found in the Grants.gov Applicants tab.

OPPORTUNITY & PACKAGE DETAILS:

Opportunity Number:	HR001118S0017
Opportunity Title:	PREventing EMerging Pathogenic Threats
Opportunity Package ID:	PKG00237724
CFDA Number:	12.910
CFDA Description:	Research and Technology Development
Competition ID:	
Competition Title:	
Opening Date:	01/19/2018
Closing Date:	03/27/2018
Agency:	DARPA - Biological Technologies Office
Contact Information:	BAA Coordinator PREEMPT@darpa.mil

APPLICANT & WORKSPACE DETAILS:

Workspace ID:	WS00094394
Application Filing Name:	Project DEFUSE
DUNS:	0770900660000
Organization:	ECOHEALTH ALLIANCE INC.
Form Name:	R & R Subaward Budget 10 YR Subform
Form Version:	1.4
Subform Name:	USGS Ntl. Wildlife Health Cen
Requirement:	Optional
Download Date/Time:	Mar 06, 2018 05:28:38 PM EST
Form State:	Error(s)

FORM ACTIONS:

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 1** **Start Date:** 10/01/2018 **End Date:** 09/30/2019

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Tonie		Rocke		129,590.00	1.00			10,800.00	3,329.00	14,129.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Coordinates animal studies										

Additional Senior Key Persons: [Add Attachment] [Delete Attachment] [View Attachment]

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 91,105.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00		24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel: 24,782.00

Total Salary, Wages and Fringe Benefits (A+B): 115,887.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	5,000.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	21,184.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.		
10.		
	Total Other Direct Costs	33,784.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	154,671.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	154,671.00	99,829.00

Total Indirect Costs 99,829.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	254,500.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	254,500.00

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 2** **Start Date:** 10/01/2019 **End Date:** 09/30/2020

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Tonie		Rocke		129,590.00	1.00			10,800.00	3,329.00	14,129.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Coordinates animal studies										

Additional Senior Key Persons: [Add Attachment] [Delete Attachment] [View Attachment]

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 91,105.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00		24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel: 24,782.00

Total Salary, Wages and Fringe Benefits (A+B): 115,887.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	8,000.00
2. Foreign Travel Costs	
Total Travel Cost	8,000.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	18,184.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	12,600.00
9.		
10.		
	Total Other Direct Costs	30,784.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	154,671.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	154,671.00	99,829.00

Total Indirect Costs 99,829.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	254,500.00

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	254,500.00

L. Budget Justification

(Only attach one file.) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 3** **Start Date:** 10/01/2020 **End Date:** 09/30/2021

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Tonie		Rocke		129,590.00	1.00			10,800.00	3,329.00	14,129.00
Project Role:	Co-Investigator										
Dr.	Rachel		Abbott		61,006.00	12.00			61,006.00	15,970.00	76,976.00
Project Role:	Coordinates animal studies										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 91,105.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
3	Undergraduate Students			3.00	24,782.00		24,782.00
	Secretarial/Clerical						

3 **Total Number Other Personnel**

Total Other Personnel: 24,782.00

Total Salary, Wages and Fringe Benefits (A+B): 115,887.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	8,000.00
2. Foreign Travel Costs	8,000.00
Total Travel Cost	16,000.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	13,334.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	9,450.00
9.		
10.		
	Total Other Direct Costs	22,784.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	154,671.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	154,671.00	99,829.00

Total Indirect Costs 99,829.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	254,500.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	254,500.00

L. Budget Justification

(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 4

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0389759340000 **Enter name of Organization:** USGS National Wildlife Health Center

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 4** **Start Date:** 10/01/2021 **End Date:** 04/30/2022

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Tonie		Rocke		129,590.00	1.00			10,800.00	3,329.00	14,129.00

Project Role: Co-Investigator

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Rachel		Abbott		61,006.00	6.00			30,502.00	7,986.00	38,488.00

Project Role: collate data, assist with reprots writing and publication

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 52,617.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						

Total Number Other Personnel

Total Other Personnel

Total Salary, Wages and Fringe Benefits (A+B) 52,617.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	4,525.00
2. Foreign Travel Costs	5,000.00
Total Travel Cost	9,525.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal care	
9.		
10.		
	Total Other Direct Costs	

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	62,142.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Total direct costs	64.54	62,141.00	40,108.00

Total Indirect Costs 40,108.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)
USGS National Wildlife Health Center

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	102,250.00

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	102,250.00

L. Budget Justification

(Only attach one file.) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		325,932.00
Section B, Other Personnel		74,346.00
Total Number Other Personnel	9	
Total Salary, Wages and Fringe Benefits (A+B)		400,278.00
Section C, Equipment		
Section D, Travel		38,525.00
1. Domestic	25,525.00	
2. Foreign	13,000.00	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		87,352.00
1. Materials and Supplies	52,702.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	34,650.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		526,155.00
Section H, Indirect Costs		339,595.00
Section I, Total Direct and Indirect Costs (G + H)		865,750.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		865,750.00

Re: PARC FEA

Rocke, Tonie E <trocke@usgs.gov>

Thu 3/15/2018 9:40 AM

To: Jerome.Unidad@parc.com <Jerome.Unidad@parc.com>

Hi Jerome: Thanks for the quick response. We are working through our budget to see what is possible. Perhaps we could chat later this afternoon. One thing I want to discuss is how the laboratory studies might go. The most expensive part of our work is the care and feeding of bats (they have to hand fed so it is quite labor intensive). So the sooner we can test in field settings the better. Also, we typically use rhodamine B in our preparation because it shows up in animals hair (visible under a fluorescence microscope) within 24 hours of consumption. So we can go in 1-2 days after spraying, capture animals to collect hair, and assess the rates of application to the bats. I don't know if you are aware of this dye, but it is bright fuschia, comes in powder form but is readily dissolved, and gets everywhere. The more work we can do in a field setting (versus an indoor laboratory) the better. For this project, if we can even get to a hand-held sprayer to show proof of concept, we'd be happy with that, as we believe DARPA would invest more in the project if we get that far. Let me know what you think. I am trying to loop in the PI as well but not sure he is available today. Best -Tonie

On Wed, Mar 14, 2018 at 5:46 PM, <Jerome.Unidad@parc.com> wrote:

> Hi Tonie,
>
> I agree based on our discussion that I think there's a lot of interesting space for our technology for wildlife health management that we can work on together. We are certainly interested in exploring these other funding opportunities with you, particularly the WNS one which seems to be in the intermediate term.
>
> Regarding PRE-EMPT, we understand that coming in this late that you guys probably have already fleshed out the project direction and tasks with equivalent budget and there might not be a lot of flexibility. In our proposed involvement, we would not want to change anything that you already just fleshed but rather supplement it with our spray technology. Based on preliminary estimation on how this involvement might be like, I came up with the following tasks and a lean estimate of the associated cost:
>
> Phase I
>
> 1. Development of a prototype FEA system for lab testing (Year 1) - $(b) (4)
> 2. Optimization of FEA spray conditions for PRE-EMPT fluids (Year 1) - $(b) (4)

3. Refinements of FEA delivery system (setup, fluid form ion, general aerosol delivery scheme) based on preliminary lab testing (Year 2) - $(b) (4)
4. Preliminary design of a field-deployable FEA system (Year 2) - $(b) (4)

Phase II

5. Fabrication and testing of field-deployable FE ystems (Year 3) - $(b) (4)
6. Project management and communication - $(b) (4)

The cost of the entire involvement as drafted is $(b) (4) over the full period of 3.5 years (Phase I and II). Some of the tasks proposed above (example task 3) are included to ensure that we refine our technology continually to meet the requirements of the application at hand. We have a good working relationship with DARPA on various projects that we would want to maintain and, at the same time, we would like to take this chance to work with institutions such as yours on something with broad impact.

Please let me know what your thoughts are and whether this might work. I can make myself available for a phone call tomorrow, as needed. Also, feel free to loop in your project PI/prime in the discussion in case it might be helpful.

Thanks,

Jerome

From: "Rocke, Tonie" <trocke@usgs.gov>
Date: Wednesday, March 14, 2018 at 10:45 AM
To: "Unidad, Jerome <Jerome.Unidad@parc.com>" <Jerome.Unidad@parc.com>
Subject: Re: PARC FEA

Hi Jerome: I had a good conversation with my colleagues and shared your material and video link with them. They agreed this looks like a great option for us, so we'd like to further explore how to move forward. Our PREEMPT proposal encompasses a wide variety of aims related to understanding and managing disease in bats in SE Asia, primarily in relation to SARS/coronaviruses. So a big part of the project is testing and developing the appropriate immune boosting agents. At the same time, we'd like to start developing the methods for delivery to bats via technology like your FEA you've described, first testing in laboratories and small caves - stateside, with the ultimate goal of conducting a field trial in a single cave system in China as proof of concept. So given that, what kind of budget do you think you might need to be involved, or alternatively, could you break things down for me by task, so we can see how we might structure this in stages? Honestly, we don't have alot of flexibility in the budget at the moment, but we see a ton of applications of this technology for your company in the future in managing wildlife health issues, not only with DARPA, but also DOI (white nose syndrome), USDA (bat rabies), and other government agencies, both foreign and domestic, so perhaps your company might view this as a good opportunity to test and illustrate the value of your technology. There is also another funding opportunity I am looking at for WNS that we might be able to partner on if you are

interested. Let me know what you think. Also, with your permission, we'd like to include the link to the video in our proposal and perhaps pull an illustration or two for the text of the proposal with credits of course. Best regards -Tonie

On Tue, Mar 13, 2018 at 6:48 PM, <Jerome.Unidad@parc.com> wrote:

> Yes, certainly split over 2-3 years. We can be very flexible on the workflow and I'd say we can structure it for maximal benefit of the project. This could mean developing an initial benchtop prototype quickly with some optimization as early as possible so that we can transition the setup to the corresponding partners (e.g. your group) to initiate the lab testing as soon as possible, and then spend the succeeding work on refining various aspects (fluid formulation for targeted spreading or bioefficacy, etc.) and maybe later on developing a field-deployable version, with motion-actuation (or timed-actuation, whatever case maybe), for Phase 2. We should be able to flesh it out very quickly depending on whatever structure you guys already have in mind.
>
> Best,
>
> Jerome
>
> ---
>
> Jerome Unidad, PhD
>
> Advanced Manufacturing and Deposition Systems
> Hardware Systems Laboratory
>
> PARC, A Xerox Company
>
> **From:** Rocke, Tonie [mailto:trocke@usgs.gov]
> **Sent:** Tuesday, March 13, 2018 12:24 PM
> **To:** Unidad, Jerome <Jerome.Unidad@parc.com> <Jerome.Unidad@parc.com>
> **Subject:** Re: PARC FEA
>
> Thanks Jerome. That is just what i need. The video is awesome. I'll talk things over with the PI tomorrow. In terms of a subcontract, do you think we could split it over 2-3 years? Best -Tonie
>
> On Tue, Mar 13, 2018 at 1:53 PM, <Jerome.Unidad@parc.com> wrote:
>
> > Tonie,
> >
> > Thanks for reaching out. Here's a 1-pager on our spray technology. If you are curious about how the spray might actually look like, you can check out a video here -- https://www.parc.com/services/focus-area/amds/
> >
> > We would really be interested in working with your proposal team on this. If possible, it will be more value-generating for us to be a subcontractor and to contribute more to tailoring

our spray technology for the intended use case. I'd also like to mention that another aspect we could bring to the table is in transitioning out the technology into a reality, particularly towards commercialization, because we have a good history on this – particularly on the device side but also, and increasingly, in the biomedical space through other commercial partners.

Please let me know how this might turn out.

Thanks,

Jerome

From: "Rocke, Tonie" <trocke@usgs.gov>
Date: Tuesday, March 13, 2018 at 5:11 AM
To: "Unidad, Jerome <Jerome.Unidad@parc.com>" <Jerome.Unidad@parc.com>
Subject: Re:

Hi Jerome: 1-2 CT is fine for me. I may not be in my office, however, so can I call you? Is this number good? 650-812-4209. Thanks -Tonie

On Mon, Mar 12, 2018 at 8:34 PM, <Jerome.Unidad@parc.com> wrote:

> Sorry, I mixed up the schedule. I actually do have a meeting in the 1-2PM PST slot, how about 11AM-12PM PST (1-2PM CT)?
>
> Thanks,
>
> Jerome
>
> ---
>
> **From:** "Rocke, Tonie" <trocke@usgs.gov>
> **Date:** Monday, March 12, 2018 at 5:25 PM
> **To:** "Unidad, Jerome <Jerome.Unidad@parc.com>" <Jerome.Unidad@parc.com>
> **Subject:** <no subject>
>
> Hi Jerome: I have been working on developing vaccines for use in managing disease in wild bats - e.g. rabies in vampire bats and white-nose syndrome in insectiverous bats. I am also collaborating on a PREEMPT proposal and one of my collaborators passed on your contact information and description regarding PARC's spray technology for possible

application in this field. Would you have time to chat tomorrow? I'll be available anytime after noon CT. Thanks much! -Tonie

--

Tonie E. Rocke

USGS National Wildlife Health Center

6006 Schroeder Rd.

Madison, WI 53711

608-270-2451

trocke@usgs.gov

--

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> --
>
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> 608-270-2451
>
> trocke@usgs.gov

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

Re: FW: Nanoparticle slide from Ralph

Rocke, Tonie E <trocke@usgs.gov>

Thu 3/8/2018 7:52 AM

To: Baric, Toni C <antoinette_baric@med.unc.edu>
Cc: Baric, Ralph S <rbaric@email.unc.edu>

Hi Toni (nice to meet a fellow Toni - I don't often); Yes, 11:30 works for me. Is that ET? I'm not yet sure if I'll be working from home tomorrow, so probably best if I call you. I'd like to hear your thoughts on how these proteins are best delivered to bats and what has been attempted so far. Thanks -Tonie

On Thu, Mar 8, 2018 at 7:07 AM, Baric, Toni C <antoinette_baric@med.unc.edu> wrote:

> Dear Tonie,
>
> Can you make a call at 11:30 am on March 9?
>
> Toni
>
> ---
>
> **From:** Baric, Ralph S
> **Sent:** Thursday, March 08, 2018 8:06 AM
> **To:** Rocke, Tonie <trocke@usgs.gov>
> **Cc:** Baric, Toni C <antoinette_baric@med.unc.edu>
> **Subject:** RE: FW: Nanoparticle slide from Ralph
>
> Hi Tonie, nice to hear from you. Toni will help find a time for us to talk. Ralph
>
> **From:** Rocke, Tonie [mailto:trocke@usgs.gov]
> **Sent:** Wednesday, March 7, 2018 8:37 PM
> **To:** Baric, Ralph S <rbaric@email.unc.edu>
> **Subject:** Re: FW: Nanoparticle slide from Ralph
>
> Hi Ralph: I have a couple of questions about the SARS-CoV spike glycoproteins you are developing with respect to the DARPA grant we are collaborating on. Do you have time for a call sometime tomorrow? I have unfortunately contracted the flu so I am working from home for a few days. I'd be happy to call you if you can provide me a time and number. Many thanks! -Tonie
>
> On Mon, Mar 5, 2018 at 1:55 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

Toni – this is the info from Ralph Baric on the nanoparticle work he's been involved in…

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4474

www.ecohealthalliance.org

@PeterDaszak

@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Baric, Ralph S [mailto:rbaric@email.unc.edu]
Sent: Friday, March 2, 2018 12:31 PM
To: Peter Daszak

Cc: Sheahan, Timothy Patrick
Subject: FW: Slide

The microparticle delivery system

From: Ainslie, Kristy
Sent: Wednesday, February 14, 2018 10:14 AM
To: Bachelder, Eric Michael <ebacheld@email.unc.edu>; Baric, Ralph S <rbaric@email.unc.edu>
Subject: Slide

R-

It was nice chatting with you today. I think we have a lot of mutual interests. I have composed a slide which I think highlights most of our platforms features. Let me know if you have any questions or concerns.

Thanks

K

Kristy M. Ainslie, PhD

Associate Professor - UNC Eshelman School of Pharmacy

Division Pharmacoengineering & Molecular Pharmaceutics

4211 Marsico Hall - 125 Mason Farm Rd - Chapel Hill, NC 27599

Ph: 919-962-4556

http://ainslielab.web.unc.edu

--

Tonie E. Rocke

Re: Invitation to be an advisor on a proposal we're submitting

Rocke, Tonie E <trocke@usgs.gov>

Fri 2/2/2018 12:41 PM

To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Alison Andre <andre@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>

Thanks for the call. Papers as promised. -Tonie

On Fri, Feb 2, 2018 at 12:08 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

> Can I call in about 10 mins – 1.15 my time, 12.15 yours?
>
> Cheers,
>
> Peter
>
> **Peter Daszak**
>
> *President*
>
> EcoHealth Alliance
>
> 460 West 34th Street – 17th Floor
>
> New York, NY 10001
>
> Tel. +1 212-380-4473
>
> www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Friday, February 2, 2018 1:05 PM
To: Peter Daszak
Cc: William B. Karesh; Aleksei Chmura; Alison Andre; Luke Hamel
Subject: Re: Invitation to be an advisor on a proposal we're submitting

Hi Peter: Sounds interesting. Yes, I'm available if we can talk fairly soon, say before 1:30 my time. I am heading out of town this afternoon. -Tonie

On Fri, Feb 2, 2018 at 11:57 AM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

Tonie,

We're submitting a proposal for work on bat viruses, and need some advice on vaccine delivery to wildlife. You're the world expert on this right now, and I wondered if you'd be able to talk briefly today (Friday), anytime this afternoon.

Also, I'd really like to invite you to be part of the proposal as an advisor to help with vaccine delivery. Could we talk about this also..

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

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New York, NY 10001

Tel. +1 212-380-4473

www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Rocke, Tonie [mailto:trocke@usgs.gov]
Sent: Wednesday, July 19, 2017 1:49 PM
To: Brian Baker
Cc: Peter Daszak; Anthony Ramos; Aleksei Chmura
Subject: Re: Prairie Dog Papers NWHC/EHA Press Release

Hi Brian: Yes, when the articles went online we released a media alert. Here's a link. Feel free to use whatever you would like. Best regards -Tonie

This release can be found in the USGS Newsroom at: https://www.usgs.gov/news/oral-plague-vaccine-helps-reduce-outbreaks-prairie-dog-colonies.

On Wed, Jul 19, 2017 at 12:43 PM, Brian Baker <baker@ecohealth.net> wrote:

Hi Tonie,

Peter recently attended a conference in South Korea where he spoke with Jonathan Sleeman about your group's prairie dog papers in *EcoHealth*, and decided to put together press release to make a big splash! Since they are already published online, we were going to have the press release coincide with the publishing of the print-version, accompanied by an intro piece in the Journal from Dan Salkeld.

Has the NWHC had any press out regarding your papers, or do you have anyone that EHA could collaborate with? I've cc'd Anthony Ramos, our Senior Director of Marketing and Development, as he will be the lead on this for us.

Thanks!

Brian

—

Brian Baker

Assistant Managing Editor, *EcoHealth*

460 West 34th Street, 17th Floor

New York, NY 10001

1.212.380.4498 (direct)

brian.hartman.baker (Skype)

Website: www.ecohealth.net
Submissions and Log-in: https://mc.manuscriptcentral.com/ecohealth
Author Instructions: http://www.ecohealth.net/submit.php

--

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PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Protection of bats *(Eptesicus fuscus)* against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine

Ben Stading[1], James A. Ellison[2], William C. Carson[2], Panayampalli Subbian Satheshkumar[2], Tonie E. Rocke[3‡*], Jorge E. Osorio[1‡*]

1 Department of Pathobiological Sciences, University of Wisconsin - Madison, Madison, Wisconsin, United States of America, **2** Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **3** US Geological Survey, National Wildlife Health Center, Madison, Wisconsin, United States of America

‡ These authors are joint senior authors on this work.
* jorge.osorio@wisc.edu (JEO); trocke@usgs.gov (TER)

Citation: Stading B, Ellison JA, Carson WC, Satheshkumar PS, Rocke TE, Osorio JE (2017) Protection of bats *(Eptesicus fuscus)* against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. PLoS Negl Trop Dis 11(10): e0005958. https://doi.org/10.1371/journal.pntd.0005958

Editor: Charles E Rupprecht, Wistar Institute, UNITED STATES

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Data Availability Statement: All relevant data are within the paper and its Supporting Information Files.

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Abstract

Rabies is an ancient neglected tropical disease that causes tens of thousands of human deaths and millions of cattle deaths annually. In order to develop a new vaccine for potential use in bats, a reservoir of rabies infection for humans and animals alike, an *in silico* antigen designer tool was used to create a mosaic glycoprotein (MoG) gene using available sequences from the rabies Phylogroup I glycoprotein. This sequence, which represents strains more likely to occur in bats, was cloned into raccoonpox virus (RCN) and the efficacy of this novel RCN-MoG vaccine was compared to RCN-G that expresses the glycoprotein gene from CVS-11 rabies or luciferase (RCN-*luc*, negative control) in mice and big brown bats (*Eptesicus fuscus*). Mice vaccinated and boosted intradermally with 1 x 10^7 plaque forming units (PFU) of each RCN-rabies vaccine construct developed neutralizing antibodies and survived at significantly higher rates than controls. No significant difference in antibody titers or survival was noted between rabies-vaccinated groups. Bats were vaccinated either oronasally (RCN-G, RCN-MoG) with 5x10^7 PFU or by topical application in glycerin jelly (RCN-MoG, dose 2x10^8 PFU), boosted (same dose and route) at 46 days post vaccination (dpv), and then challenged with wild-type big brown variant RABV at 65 dpv. Prior to challenge, 90% of RCN-G and 75% of RCN-MoG oronasally vaccinated bats had detectable levels of serum rabies neutralizing antibodies. Bats from the RCN-*luc* and topically vaccinated RCN-MoG groups did not have measurable antibody responses. The RCN-rabies constructs were highly protective and not significantly different from each other. RCN-MoG provided 100% protection (n = 9) when delivered oronasally and 83% protection (n = 6) when delivered topically; protection provided by the RCN-G construct was 70% (n = 10). All rabies-vaccinated bats survived at a significantly ($P \leq 0.02$) higher rate than control bats (12%; n = 8). We have demonstrated the efficacy of a novel, *in silico* designed rabies MoG antigen that conferred protection from rabies challenge in mice and big brown bats in laboratory studies. With further development, topical or oronasal administration of the RCN-MoG

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vaccine could potentially mitigate rabies in wild bat populations, reducing spillover of this deadly disease into humans, domestic mammals, and other wildlife.

Author summary

Rabies remains a significant and costly zoonotic disease worldwide. While control of canine rabies can significantly diminish the threat to human health, spillover of rabies and related lyssaviruses from bats into terrestrial animals and humans continues to be an important issue. Here we describe the development of a novel rabies vaccine, using raccoonpox virus (RCN) as a viral vector, and a computer designed rabies virus mosaic antigen. We demonstrate that this new vaccine leads to protection against experimental challenge in wild caught big brown bats when administered oronasally or topically. This technology could be adapted to target other bat species and also be directly applicable toward control of vampire-bat associated rabies in Mexico and Central and South America.

Introduction

Rabies is a fatal viral zoonotic disease known to humans for nearly four millennia that continues to cause significant public health concern with over 50,000 human deaths every year [1]. Fortunately, over 15 million people receive post-exposure prophylaxis for rabies exposure, which effectively prevents rabies if administered promptly [2]. In Mexico and Central and South America, rabies transmitted by vampire bats is a tremendous public health and economic issue, as it threatens not only the people in these areas, but also an at-risk population of more than 70 million head of cattle [3–6]. Vampire bats were thought to have caused cattle losses in Latin America worth more than $40 million US in 1983, and again in 1984 [7], and these losses, coupled with the cost of measures to prevent bovine rabies, are a significant economic burden.

Rabies virus (RABV, Family: *Rhabdoviridae*, Genus: *Lyssavirus*) has adapted to numerous mammalian reservoirs that maintain transmission, typically by bite, and as a result has evolved into specific lineages and variants. Bats are considered the primary evolutionary host of RABV [8] and harbor a diversity of other lyssaviruses, all of which cause rabies disease, with non-RABV lyssaviruses occurring in the Old World and Australia [9,10]. Lyssaviruses are divided into distinct phylogroups based on serological analysis and genome sequence [11]. While lyssaviruses within phylogroup I (PG-I) are considered cross-protective immunologically, epidemiologically important antigenic variation between vaccine strains and wild-type rabies viruses have been observed [12] and variable vaccine efficacy has been reported against some PG-I viruses[13]. In addition, numerous antigenic variants of rabies have been found in bats in the Americas [14]. In Brazil, nine different variants have been reported; in Mexico, at least 7, and antigenic variants differ between bats species and geographic locations.

Rabies in terrestrial wild mammals can be successfully controlled, and in some areas, eliminated through the use of oral rabies vaccination (ORV) campaigns [15–17], but similar mass vaccination has not yet been attempted for wild bats. Recombinant viral-vectored vaccines have been developed to make use of the antigenicity of the RABV surface glycoprotein (G). The main benefit of these viral-vectored constructs is their ability to induce immunity when given orally, which makes them effective and efficient for vaccinating wildlife. A vaccinia virus

construct expressing the G protein (or V-RG) has been used extensively for wild carnivores, but this construct can cause vaccinia infection in humans that are inadvertently exposed to the vaccine, especially in immuno-compromised individuals [18–20]. More recently a similar vaccine has been developed and licensed using a human adenovirus vector (ONRAB) [21], but to our knowledge, that vector (and vaccine) has not yet been tested in bats.

Our previous study showed that RCN is a suitable vaccine vector for bats; it safely expressed exogenous antigens and induced significant immune responses following mucosal exposure of *Tadarida brasiliensis* bats [22]. The safety profile of the RCN vector has been evaluated previously [23–25], and a RCN-based sylvatic plague vaccine is under evaluation in field trials in prairie dog populations [26]. In this study, we used G sequences from 664 RABV to design a novel PG-I lyssavirus mosaic glycoprotein gene (MoG) that could potentially provide broader antigenic coverage for the variety of rabies strains circulating in bats, and perhaps a more effective vaccine. We successfully expressed MoG in the RCN vaccine vector and then evaluated its efficacy in preventing rabies mortality in mice and big brown bats (*Eptesicus fuscus*) in laboratory challenge studies, comparing it to a previously reported RCN-G construct that expresses the CVS-11 glycoprotein [27]. Our results suggest that MoG is a successful rabies antigen as both mucosal and topical application of RCN-MoG protected against high-dose rabies virus challenge.

Methods

Cells and viruses

Recombinant viruses were generated and amplified on cell monolayers of rat embryonic fibroblasts (Rat-2, ATCC #CRL-1764) or African Green monkey (*Chlorocebus sabaeus)* kidney epithelial cells (BSC40, ATCC #CRL-2761, or Vero, ATCC #CCL-18). Cell cultures were maintained at 37˚C and 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) or Opti-MEM (Life technologies, Madison, WI 53719), supplemented with 2–5% fetal bovine serum (FBS). Recombinant RCN-G [3] and wild-type RCN (RCN-wt) viruses were provided by the Centers for Disease Control (CDC), Atlanta, GA, while the RCN-luc strain used in this study was previously described [28].

The RABV CVS-11 (GenBank accession no AB069973) strain used in mouse challenge studies was provided by the Wisconsin State Laboratory of Hygiene and was amplified on baby hamster kidney cells (BHK-21, ATCC #CCL-10) in DMEM as described elsewhere [29]. The virus was titered by infecting BHK-21 cells in 96-well plates with serial dilutions in quadruplicate. After 72 hours, the cells were fixed with 80% acetone and subsequently probed with a FITC-conjugated rabies antibody (LIGHT DIAGNOSTICS Rabies DFA Reagent 5100, Millipore, Billerica, Massachusetts, USA) to determine focus forming unit (FFU) titer.

The wild type big brown bat variant RABV used for bat challenge has been previously described (GenBank #JQ685920.1); it was isolated from the salivary glands of a naturally infected big brown bat in Pennsylvania during 2006 and subsequently passaged once through murine neuroblastoma cell culture [30]. The virus was provided under a cooperative research and development agreement with the CDC (A06-3684).

Design and construction of recombinant RCN-MoG virus

Design and *in silico* assessment of mosaic rabies glycoprotein. All available sequences for PG-I lyssaviruses (rabies virus, Duvenhage virus, European bat lyssavirus -1 and -2, Aravan virus, Australian bat lyssavirus, Khujand virus, Irkut virus, and Bokeloh bat lyssavirus) were obtained from the National Center for Biotechnology Information (NCBI) and were screened to exclude incomplete and redundant sequences. As a result, a total number of 664

glycoprotein sequences were submitted to the Mosaic Vaccine Designer tool webpage (http://www.hiv.lanl.gov/content/sequence/MOSAIC/makeVaccine.html) to generate a mosaic protein sequence as previously described [31]. Mosaic proteins are assembled *in silico* from fragments of the natural proteins using a genetic algorithm in a way that prevents formation of new epitopes. The program chooses the most frequent epitopes and combines them to form a synthetic antigen, unlike consensus sequences which pick the most frequent amino acid at each position. The parameter options were set as follows: 1) the cocktail size was set to 1 to generate a single peptide that represented all input glycoproteins, 2) the rare threshold was set to 3 for optimal value, and 3) the epitope length parameter was set to an amino acid length of 12-mer in an attempt to match the length of natural T helper cell epitopes. The resulting mosaic lyssavirus glycoprotein was back-translated, codon optimized for expression in vaccinia virus, and then commercially synthesized (GenScript USA Inc., Piscataway, NJ, USA).

Sequences, along with the optimal mosaic vaccine candidate (MoG), were aligned with default settings in muscle v3.8.31 [32] with subsequent manual correction and curation in Mesquite [33]. A maximum likelihood tree was inferred using IQ-TREE v1.4.2 [34] employing the best-fit model of molecular evolution as determined by the automatic model selection procedure (data available upon request). Statistical support values were determined using the ultrafast bootstrap algorithm (n = 1000; [35]) and SH-like approximate likelihood ratio tests (n = 1000; [36]).

Construction of recombinant RCN viruses. To aid in the selection of recombinant RCN constructs, we first created an RCN virus with the thymidine kinase (*tk*) gene knocked-out and replaced with green fluorescent protein (GFP). Removal of the *tk* gene results in attenuation of poxviruses without loss of immunogenicity [37], and also serves as a good insertion site for heterologous genes. The RCN-*tk*$^{-}$GFP construct was generated by homologous recombination as described elsewhere[38]. Briefly, Vero cells were co-transfected with RCN-*wt*, at a multiplicity of infection (MOI) of 0.05 PFU/cell, and the pTK-GFP plasmid using the FuGENE HD transfection reagent (Promega Corp., Madison, WI, USA). GFP-positive plaques were then selected through 5 rounds of viral purification.

For creating RCN-MoG, the MoG sequence was cloned into the multiple-cloning site (MCS) in the pTK vector under control of the SE/L promoter, and then positive clones were selected. The RCN-MoG construct was then generated by co-transfecting the pTK-SE/L-MoG plasmid and RCN-*tk*$^{-}$GFP in BSC-40 cells as described above.

An additional construct was made that utilized an internal ribosomal entry site (IRES) for the expression of the MoG antigen, as it has been found to enhance expression in other constructs [39]. The RCN-IRES-MoG was constructed using the same methods as above by creating a pTK-SE/L-IRES-MoG.

***In vitro* expression of RCN-MoG construct.** Immunofluorescence and western blot analysis were used to confirm the expression of the artificial MoG antigen by the RCN construct. For immunofluorescence, 6-well plates of Vero cells were infected with RCN-G, RCN-MoG, RCN-IRES-MoG, or RCN-GFP (as a negative control) at an MOI of 1.0 PFU/cell. After 24 hours (h), the plates were fixed with 4% formaldehyde for 10 minutes (min), washed with PBS, then permeabilized with a PBS/0.2%Triton-X-100/0.2% BSA solution for 10 min on ice. The plates were then rinsed and blocked with a PBS/0.02% Triton-X-100/3% BSA solution for 30 min. After blocking, the plates were stained with a 1:1000 dilution of mouse anti-rabies Ab (Rab-50, Invitrogen, Thermo Fisher Scientific Inc., Fitchburg, WI, USA) in blocking solution overnight at 4˚C. Primary Ab was then removed, and the wells were washed four times, 10 min each, with a PBS/0.02% Triton-X-100/1.5% BSA washing solution. A secondary Ab solution with a 1:2000 dilution of Alexa Fluor 594 tagged goat anti-mouse Ab (Invitrogen, Thermo Fisher Scientific Inc.) was then added to the wells and left at room temperature for 2 h,

followed by an additional four rounds of 10 min washes with the washing solution. Wells were then observed under a fluorescence microscope (excitation wavelength: 590 nm, emission wavelength: 617 nm; AMG $EVOS_{fl}$, Thermo Fisher Scientific Inc.).

For western blot analysis, Vero cells were plated into six-well plates and infected at an MOI of 10 PFU/cell with RCN-MoG, RCN-IRES-MoG, RCN-G, or RCN-*luc* as a negative control. Cells and supernatant were collected 48 h post inoculation and lysed with Laemmli sample buffer (Bio-Rad, Hercules, CA, USA) and heated to 95˚C for 5 min. Protein was fractionated via SDS-PAGE and transferred onto a nitrocellulose membrane. Pooled serum from rabies-vaccinated mice (IMRAB3, Merial, Athens, GA, USA) was used as the primary antibody for rabies glycoprotein detection. 3,3',5,5'-Tetramethylbenzidine (TMB) was used to visualize the glycoprotein in the membranes.

Animal studies

Ethics statement. This study was carried out in strict accordance with recommendations set forth in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* [40]. All animals and animal facilities were under the control of the School of Veterinary Medicine with oversight from the University of Wisconsin (UW) Research Animal Resource Center. The protocols were approved by the UW Animal Care and Use Committee (approval #'s V01605, V005278), and studies were conducted first in mice, followed by bats.

All animal studies involving rabies virus were conducted under ABSL-2+ conditions in limited access facilities; all individuals involved in the study had documented evidence of pre-existing rabies prophylaxis through recent (<2 years) completion of the full recommended vaccination schedule or confirmation of sufficient circulating rabies neutralizing antibody titers ($\geq$0.5 IU/mL).

Mouse challenge study. A/J mice (4 week old) were purchased from Jackson Laboratory (JAX, Sacramento, CA, USA) and housed at the UW Charmany Instructional Facility according to UW husbandry protocols. After a 1 week acclimation period, the mice were separated into 4 treatment groups of n = 16 mice each. Each treatment group was vaccinated with RCN-MoG, RCN-IRES-MoG, RCN-G, or RCN-*luc* via intradermal injection of 1x 10^7 PFU's given in 30 μl in the hind limb footpad. Mice were then bled via maxillary lance every 15 days until the rabies challenge, and serum was stored at -80˚C. At 75 dpv, mice were boosted with the same vaccine and the same route as previously described. At 208 dpv (133 days post boost), six mice from each group were challenged with 25 x LD_{50} of CVS-11 RABV in 30μl via the intracerebral (IC) route. Mice were then weighed daily and euthanized if they had lost more than 20% of their maximum body weight, or if clinical signs of rabies were evident. All carcasses were frozen at -80˚C for diagnostic assessment. The study was ended 14 days after challenge.

Bat challenge study. Adult *E. fuscus* bats (N = 39) were wild-caught using mist nets in Lee county, Alabama, by Dr. Matthew Grilliot of Troy University under collection permit #8565 provided by the Alabama Department of Conservation and Natural Resources. After acclimation to captive conditions for 4 weeks, the bats were transferred to UW Charmany Instructional facility, where all vaccine studies were conducted. Upon transfer, bats were maintained in screen flight cages (Reptarium, Apogee, Dallas, TX, USA) for a quarantine period of 30 days. During this time blood samples were taken for rabies serology as described below (all bats were negative on intake), and bats were treated topically for parasites with selamectin (Zoetis, Florham Park, NJ, USA). Electronic microchip identification units (Avid Identification Systems, Inc., Folsom, Louisiana, USA) were inserted into each animal, between the scapulae, via subcutaneous injection. Bats were maintained on mealworms (*Tenebrio molitor*),

supplemented with vitamins and an omega fatty acids mixture, and water was available *ad libitum*. They were individually weighed at least once per week.

Four bats failed to adapt to captivity and died during quarantine. Two additional bats that continued to lose weight after the quarantine period died 28 days after initial vaccination, and were subsequently tested and found to be rabies negative. The remaining 33 bats formed 4 treatment groups. Three groups of females received $5x10^7$ PFU of RCN-MoG (n = 9), RCN-G (n = 10), or RCN-*luc* (n = 8) via the oronasal (ON) route, with 50μl given orally and 10μl deposited in each nostril (70 μl total volume). One group of males (n = 6) received $2x10^8$ PFU of RCN-MoG mixed with laboratory grade glycerin jelly (Carolina Biological Supply, Burlington, NC, USA) to a final volume of 250μl. This aliquot was distributed equally in the fur of the ventral lateral thorax (near the wing membrane). All bats were anesthetized for inoculation for ~5 minutes and then returned to their cages for recovery. Bats received a booster immunization (same dose and route) at 46 days post initial immunization. All bats were bled via the interfemoral vein on days 0, 21, and 65 dpv. At 65 dpv, bats were challenged with $1x10^{5.5}$ $MICLD_{50}$/ml of RABV in 100μl delivered bilaterally into the masseter muscles (50μl each). Following challenge, all bats were monitored daily for evidence of disease and weighed twice a week. Any bats that lost ≥20% of their body weight within 7 days or that had evidence of clinical rabies were euthanized under anesthesia by cardiac exsanguination, followed by administration of sodium pentobarbital (Beuthenasia-D, Intervet/Merck Animal Health, Madison, NJ, USA). Carcasses were kept at -80˚C until analysis. The study was ended 42 days post challenge, after a 14-day period with no deaths.

Rabies diagnosis and serology. Serum rabies neutralizing antibody (RVNA) titers were determined using a microneutralization test that is based on the rapid fluorescent focus inhibition test [40], with some modifications [41]. To determine RVNA titer of individual bats and mice, ten microscopic fields per well on a 4-well slide were scored for presence/absence of at least one fluorescent focus. Endpoint titers were calculated by the Reed-Muench method and were converted to international units (IU/mL) by comparison to a standard rabies immune globulin (SRIG) control containing 2 IU/mL[41]. For the objective of this study, positive RVNA titers (≥0.06 IU/mL) were defined by at least 50% neutralization of the RABV challenge virus dose (50 focus forming doses) at a 1:10 dilution. Final titers less than 0.06 IU/mL were considered negative for the presence of RVNA for the purposes of this investigation.

All mouse and bat carcasses were analyzed for evidence of rabies disease. Brain impressions were fixed in acetone at -20˚C, and RABV antigens were detected by the direct fluorescent antibody test (dFA), using fluorescein isothiocyanate (FITC)-labelled monoclonal antibody (mAb) conjugate (Fujirebio Diagnostics, Inc., Malvern, PA, USA) as described [42].

Statistical analysis. One-way analysis of variance (ANOVA) was used to analyze neutralizing antibody titers between groups of animals. Wilcoxon matched pairs T-tests were used to compare group body weights over time. Kaplan Meier survival analyses were performed to compare survival between vaccinates and controls. Probability values of 0.05 were considered significant. GraphPad Prism (v6) software (La Jolla, CA, USA) was used for all statistical analyses.

Results

Characterization of mosaic constructs

The antigenic coverage of the designed MoG sequence (S1) achieves 61% exact matches of putative T cell epitopes with an epitope length set to 12 amino acids (Fig 1A). This improves to 84% matches if 1 of those 12aa is allowed to be a mismatch (off-by-1) and 92% for off-by-2. This is similar to the results for previously described, effective mosaic proteins[43,44]. If the nominal epitope length is set to 9 amino acids, the coverage increases to 67% exact matches;

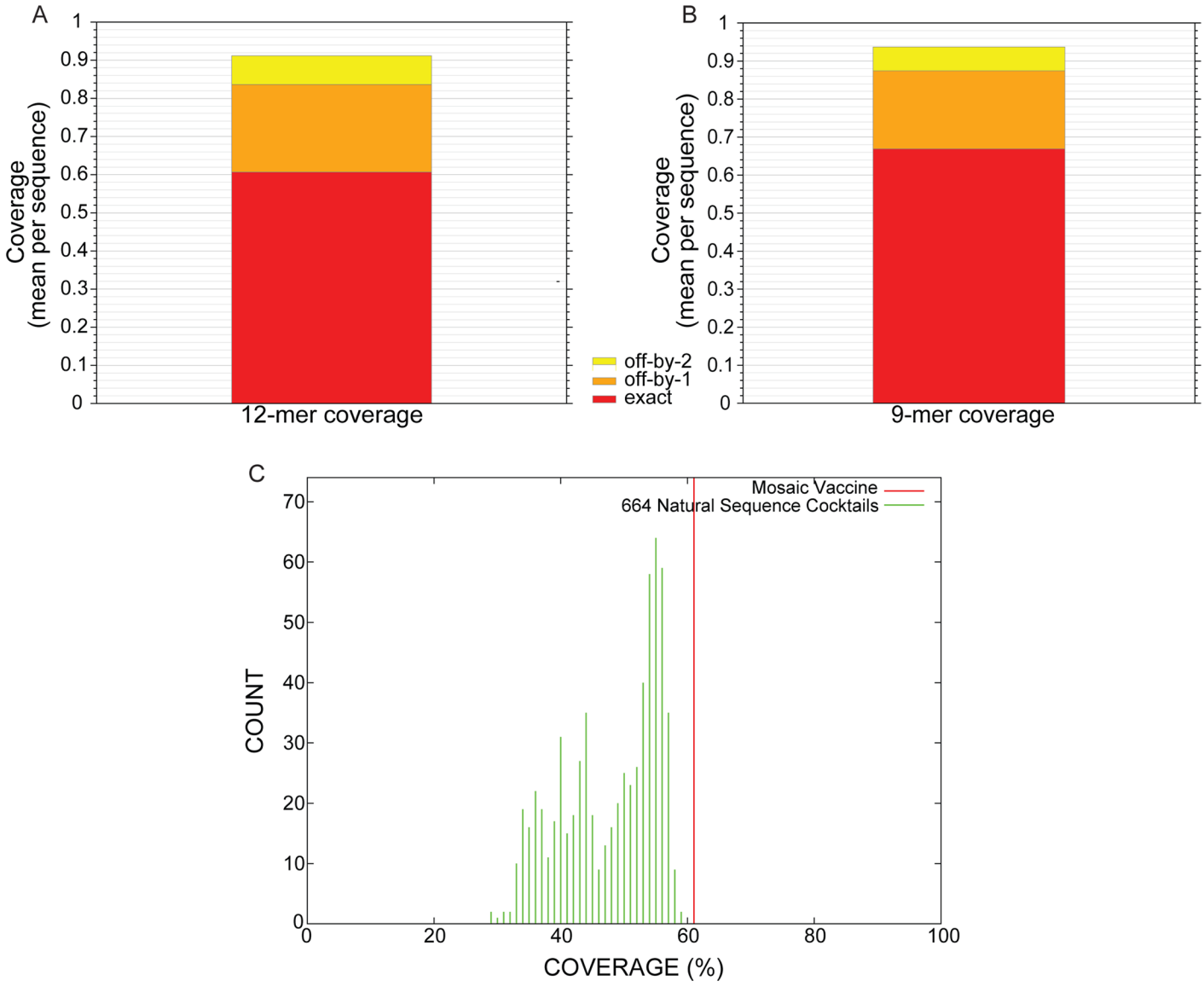

Fig 1. Antigenic coverage of putative T cell epitopes by the designed mosaic phylogroup I lyssavirus glycoprotein. A) Antigenic coverage with the epitope length set to 12 amino acids. B) Antigenic coverage with the epitope length set to 9 amino acids. C) Comparison of 12-mer epitope coverage between the mosaic sequence and all input sequences.

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87% off by 1; 94% off by 2 (Fig 1B). Comparing epitope coverage of the MoG to the other PG-I lyssaviruses used for its design, it is better than any single "wild type" virus (Fig 1C). A comparison of amino acid sequences of four major and one minor PG-I antigenic sites reveal that MoG retains most RABV sequences (Table 1).

Immunofluorescence assays of cultured cells infected with RCN-MoG, RCN-IRES-MoG, and RCN-G confirmed presence of rabies virus antigen when compared to the RCN-GFP negative control (Fig 2A). Western blot analysis revealed bands visible at ~60kDa in the pellet of the RCN-MoG, RCN-IRES-MoG, and RCN-G infected cells, and absent in the negative control, demonstrating expression of an antigenic glycoprotein (Fig 2B). The RCN-IRES-MoG seems to be slightly smaller and have a secondary band, which may indicate variation in glycosylation [46] or production of truncated forms of the MoG.

Table 1. Amino acid sequence of major phylogroup I lyssavirus antigenic sites based on Evans et al. 2012[45], including mosaic G. The underlined residues are those that differ from the RABV sequence, given in the top row.

Virus	Site IIb (34–42)	Site IIa (198–200)	Site I (226–231)	Site IV (263–264)	Site III (330–338)	Site 'a' (342–343)
RABV	GCTNLSEFS	KRA	KLCGVL	FH	KSVRTWNEI	KG
ABLV	GCTSLSGFS	KKA	KLCGIS	FN	KSVRTWDEI	KG
ARAV	GCTNLSGFT	KKA	KLCGVM	FH	KSVREWTEV	KG
BBLV	GCTTLTVFS	KKA	KLCGVS	FH	KSIRQWTEI	KG
DUVV	GCTTLTPFS	KKA	RLCGIS	FH	KSVREWKEI	KG
EBLV-1	GCTTLTPFS	KKA	RLCGVP	FH	KSVREWKEV	KG
EBLV-2	GCTTLTVFS	KKA	KLCGIS	FH	KSIREWTDV	KG
IRKV	GCTTLTAFN	KKA	KLCGMA	DR	KSIREWKEI	KG
KHUV	GCTTLSGFT	KKA	KLCGVS	FH	KSIREWSEI	KG
MoG	GCTNLSGFS	KRA	KLCGVL	FH	KSVRTWNEI	KG

https://doi.org/10.1371/journal.pntd.0005958.t001

Immunogenicity of RCN-vectored MoG vaccines and survival upon challenge in mice

Serum samples from mice (n = 16 per group) were tested by the RFFIT assay (at CDC). All RCN-rabies constructs induced significant antibody titers when measured at 45 dpv (Fig 3). No significant differences in antibody levels were observed between groups (P = 0.399).

Following rabies virus challenge, one mouse each from the RCN-G and RCN-*luc* groups were euthanized due to loss of ≥ 20% of their body weight within 3 days post challenge (dpc;

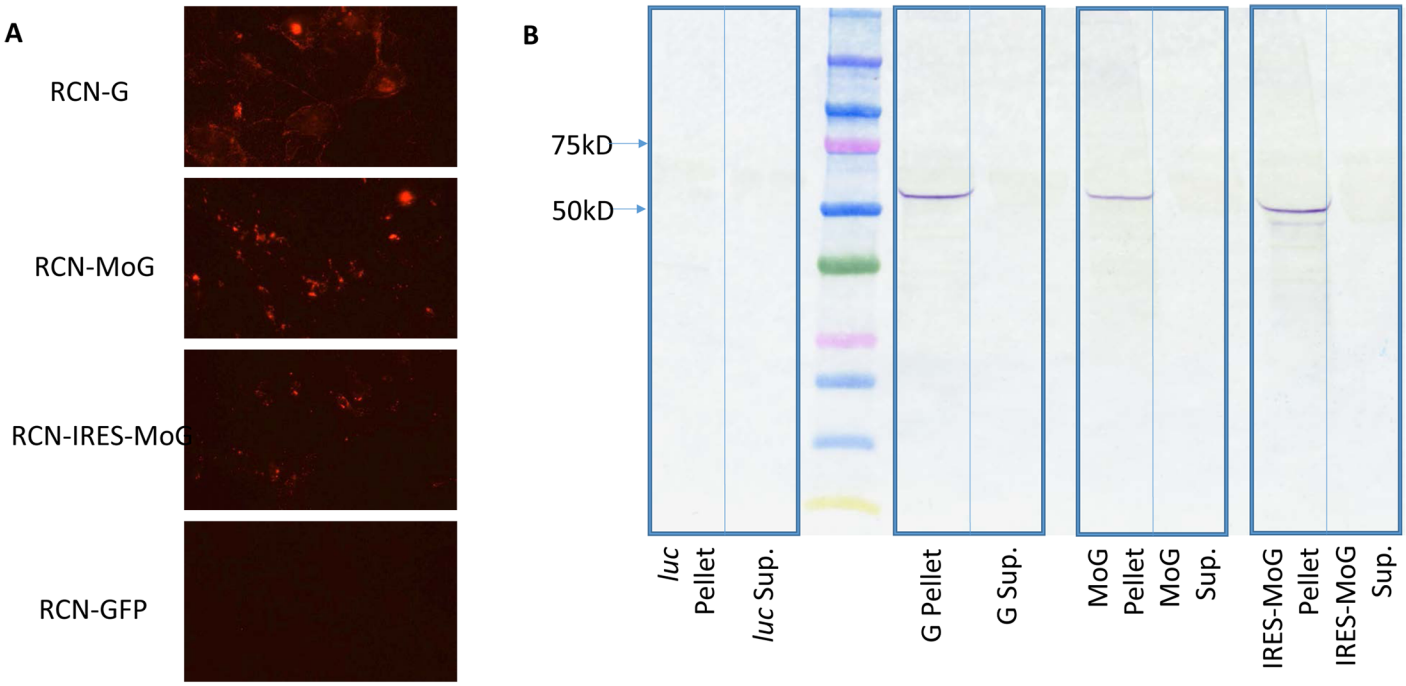

Fig 2. *In vitro* assessment of rabies glycoprotein expression in novel RCN-vectored rabies vaccines. A) Immunofluorescence of RCN expressing *in silico* designed lyssavirus phylogroup I glycoprotein (MoG) with and without an internal ribosomal entry site (IRES). A previously described RCN construct expressing the glycoprotein from rabies CVS-11 (RCN-G) was used as a positive control, and RCN expressing green fluorescent protein (GFP) was used as a negative control. B) Western blot of supernatant (Sup.) or pellet collected from Vero cells infected with RCN-MoG, RCN-IRES-MoG, RCN-G (positive control) or RCN-*luc* (negative control), The rabies glycoprotein is expected to be around 62 kDa.

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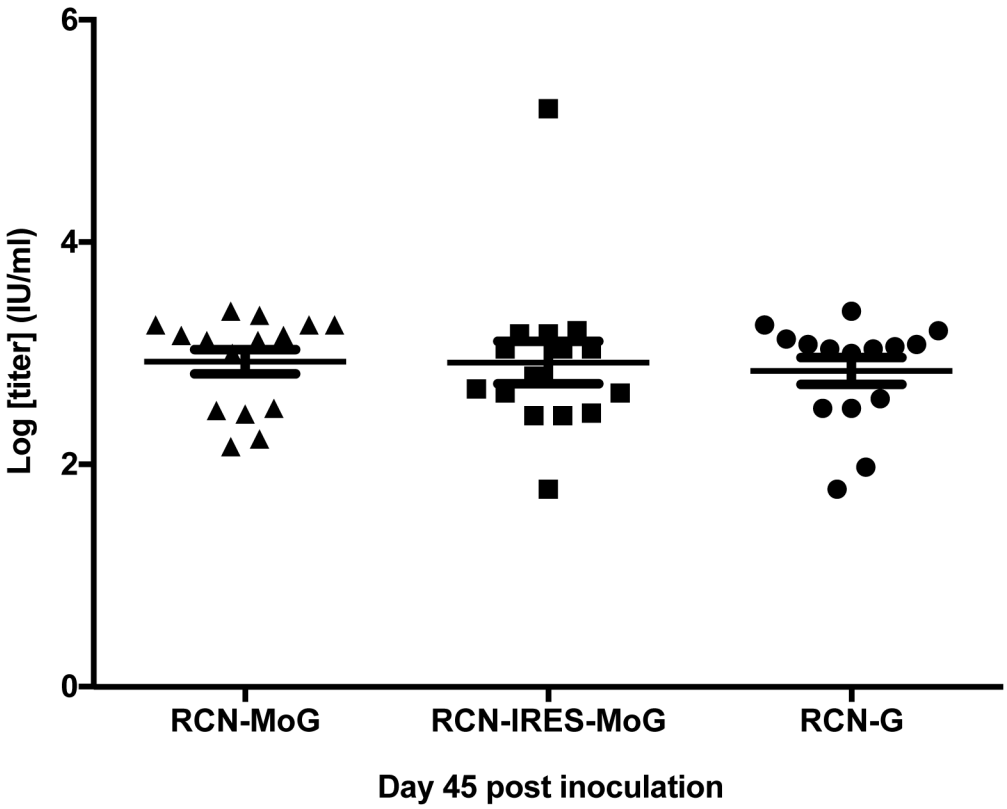

Fig 3. Rabies neutralizing antibody levels in mice following vaccination with various RCN-vectored rabies vaccines. Serum titers of rabies neutralizing antibodies (IU/ml) in mice 45 days post vaccination with RCN-MoG, RCN-IRES-MoG, or RCN-G. No significant differences were detected between groups (P = 0.399).

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Table 2). Two other mice, one in each of the RCN-G and RCN-IRES-MoG groups were found to have lost ≥ 20% of their body weight by14 dpc, the last day of the trial. All four of these mice were rabies negative by the dFA test (Table 2) and were censored in the survival analysis. All other mice that were euthanized with signs of disease during the challenge were positive by dFA. All RCN-rabies treatment groups had statistically higher survival than the RCN-*luc* negative controls ($P<0.03$). All mice survived to day 14 in the RCN-MoG group compared to 50% (3/6) in the RCN-IRES-MoG group, 80% (4/5) in the RCN-G group and 0/5 in the RCN-luc group. Although no significant difference ($P>0.05$) in survival was detected between groups that received the three rabies vaccines (Fig 4), RCN-IRES-MoG was not included in further studies in bats.

Immunogenicity of RCN-vectored vaccines and survival upon challenge in bats

After inoculation with RCN-vectored vaccines, no signs of clinical disease were evident in any of the bats. Topically vaccinated bats also showed no evidence of adverse effects due to the glycerin jelly application or the vaccine virus. No significant change in weight was evident in the groups after initial vaccination or boost ($P>0.05$, S1 Fig).

After initial vaccination, 2/9 bats from the RCN-MoG ON group had titers between 0.1–0.4 IU/ml and 4/10 bats from the RCN-G ON group responded with titers >0.5 IU/ml, while no

Table 2. Survival and % change in weight of vaccinated mice prior to and following challenge with rabies virus.

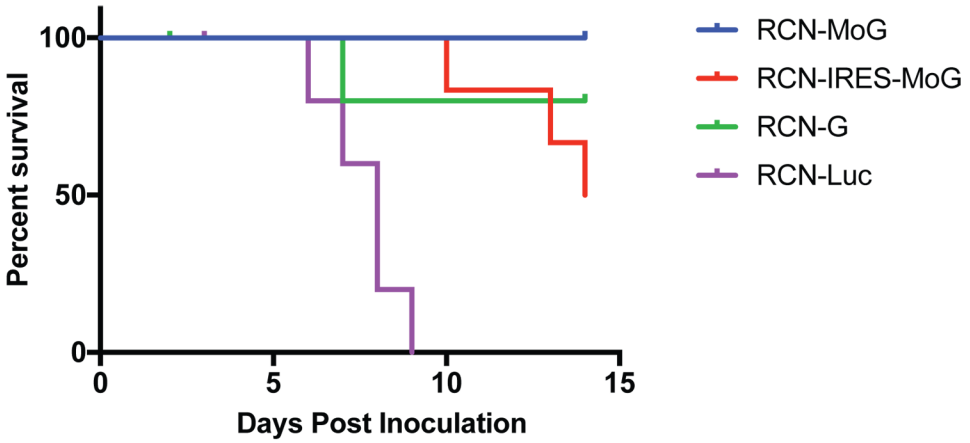
Group	Mouse ID	Day of death or euthanasia	% weight change	Challenge outcome	dFA rabies diagnosis
RCN-MOG	1		1.04	Survived	Negative
	2		1.02	Survived	Negative
	3		1.03	Survived	Negative
	4		1.00	Survived	Negative
	6		0.99	Survived	Negative
	8		0.97	Survived	Negative
RCN-IRES MOG	1	14	0.79	Died	Positive
	2		1.02	Survived	Negative
	3	13	0.79	Died	Positive
	4	10	0.77	Died	Positive
	5		0.90	Survived	Negative
	6	14	0.77	Censored	Negative
RCN-G	1	8	0.77	Died	Positive
	3		1.00	Survived	Negative
	4		0.92	Survived	Negative
	5	3	0.68	Censored	Negative
	6		1.06	Survived	Negative
	7	14	0.79	Censored	Negative
RCN-luc	9	9	0.80	Died	Positive
	10	3	0.80	Censored	Negative
	11	7	0.80	Died	Positive
	13	8	0.75	Died	Positive
	14	8	0.76	Died	Positive
	15	6	0.77	Died	Positive

https://doi.org/10.1371/journal.pntd.0005958.t002

detectable antibodies were found in any of the bats from the RCN-*luc* group or the RCN-MoG topically vaccinated bats (Table 3, Fig 5). After boost, 2/8 bats tested in the RCN-MoG ON group had titers $>$ 0.5 IU/ml, and an additional 4 bats had titers of 0.1–0.4. In the RCN-G ON group, 6/10 bats had RVNA levels $\geq$ 0.5 IU/ml, 3 had levels of 0.1–0.4, and one bat had no detectable RVNA. Even though more bats that received RCN-G ON had RVNA titers compared to RCN-MoG ON, no significant difference in titer was detected between these groups ($P = 0.22$). Bats in the RCN-*luc* and RCN-MoG topically vaccinated groups had no detectable neutralizing antibodies prior to challenge.

After challenge with rabies virus, all vaccine treatment groups had significantly greater ($P \leq 0.02$) rates of survival than the negative control (RCN-*luc*) group (Fig 6). The first confirmed rabies deaths occurred at 12 dpc and the final at 27 dpc. The majority of mortalities occurred between 12 and 19 dpc. All bats administered RCN-MoG by the ON route survived challenge, although interestingly only 2/8 had pre-challenge RVNA levels above 0.5 IU/ml (Table 2, Fig 5). Likewise, 5/6 (83%) of the bats that received RCN-MoG topically survived challenge, despite none having seroconverted. Comparatively, 7/10 of the RCN-G ON vaccinated group survived challenge, including two bats with antibody titers below 0.5 IU/ml. Interestingly, one bat in this group with a titer of 0.5 IU/ml succumbed to rabies challenge and 1/8 bats immunized with RCN-*luc* survived challenge.

No clinical signs were observed in any of the surviving bats. Direct FA confirmed rabies diagnoses consistent with our survival analysis (Table 2). All bats that were found dead or euthanized were rabies positive, while all remaining bats at the study end were negative.



P-values:	RCN-MoG	RCN-IRES-MoG	RCN-*luc*
RCN-G	0.273	0.421	0.022
RCN-MoG		0.055	0.001
RCN-IRES-MoG			0.001

Fig 4. Survival after rabies challenge in mice. Efficacies of raccoon poxvirus (RCN) vectored rabies vaccines in mice after intracerebral challenge with the CVS-11 strain of rabies virus. Every mouse (6/6) in the RCN-MoG group survived challenge to day 14 compared to 3 of 6 in the RCN-IRES-MoG group, and 4 of 5 in the RCN-G group. All (5/5) negative controls (RCN-*luc*) succumbed by day 9 post challenge. A chart of p-values associated with the survival curve is also provided. Survival of all vaccinated mice was significantly higher ($P < 0.05$) than negative controls, but there was no significant difference ($P > 0.05$) between vaccine treated groups.

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Discussion

Rabies spillover from wildlife, particularly by vampire bats (*Desmodus rotundus*), continues to be an important public health and economic issue in Mexico and Central and Latin America [17,47], despite using culling of bats as a control measure[48–50]. In this study, we demonstrated that an *in silico* designed mosaic lyssavirus PG-I glycoprotein (MoG) is an effective immunogen against rabies in mice and bats. Furthermore, a recombinant RCN-vectored vaccine expressing MoG, delivered by mucosal or topical routes, protected bats against rabies challenge. While survival did not differ significantly among any of the vaccine treated groups ($P = 0.08$), RCN-MoG provided 100% protection in ON immunized bats challenged with a wild-type big brown bat RABV variant. As in our previous study [22], both RCN vaccine constructs were safe; no evidence of morbidity was observed in treated bats. Though these results are very promising, additional challenge studies with other bat RABV variants, are needed to assess whether our bioinformatically designed RCN-MoG vaccine is an improvement over RCN-G.

Currently available rabies vaccines, which are almost entirely developed from lab-adapted strains (e.g. CVS-11), are considered protective against all PG-I lyssaviruses when given at the recommended dose and schedule. However, antigenic variation in PG-I strains has been identified and may lead to inconsistent protection [12,13]. The CVS-11 strain has been passaged over a thousand times in rabbit and mouse brains and cell culture [51]. One study showed that 5.1 units of antigenic difference exists between CVS-11 and "wild type" RABV strains isolated from different hosts, equivalent to a more than 10-fold dilution in antibody titer[12]; thus higher titers are needed for protection. For wildlife consuming variable doses of vaccines via

Table 3. Serological and survival results of vaccinated *E. fuscus* prior to and following challenge with rabies virus.

Group	Bat ID	Rabies virus titer VNA (IU/ml) days post primary inoculation		Challenge outcome	dFA rabies diagnosis
		22	65		
RCN-MoG (Oronasal)	1504	0.0	0.1	Survived	Negative
	1511	0.0	0.1	Survived	Negative
	1518	0.0	0.5	Survived	Negative
	1525	0.1	0.4	Survived	Negative
	1526	0.0	0.0	Survived	Negative
	1528	0.0	0.0	Survived	Negative
	1530	0.4	12.2	Survived	Negative
	1531	0.0	no sample	Survived	Negative
	1535	0.0	0.1	Survived	Negative
RCN-MoG (Topical)	1512	0.0	0.0	Survived	Negative
	1523	0.0	0.0	Survived	Negative
	1527	0.0	0.0	Survived	Negative
	1529	0.0	0.0	Died	Positive
	1536	0.0	0.0	Survived	Negative
	1537	0.0	0.0	Survived	Negative
RCN-G (Oronasal)	1506	13.0	12.2	Survived	Negative
	1507	3.5	12.2	Survived	Negative
	1508	0.0	0.1	Survived	Negative
	1517	0.0	0.1	Died	Positive
	1519	11.4	10.0	Survived	Negative
	1520	0.0	0.0	Died	Positive
	1522	0.0	0.5	Died	Positive
	1524	0.0	0.7	Survived	Negative
	1532	14.1	12.2	Survived	Negative
	1534	0.0	0.1	Survived	Negative
RCN-luc (Oronasal)	1501	0.0	0.0	Died	Positive
	1509	0.0	0.0	Died	Positive
	1513	0.0	0.0	Died	Positive
	1514	0.0	0.0	Survived	Negative
	1515	0.0	0.0	Died	Positive
	1521	0.0	0.0	Died	Positive
	1533	0.0	0.0	Died	Positive
	1538	0.0	0.0	Died	Positive

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the oral route of delivery, it is important to use the most efficient vaccine, protective at the lowest titer possible with the fewest doses, as boosts are generally unfeasible. Although bats were boosted in our initial study to optimize their response, testing of a single dose application will be critical in future studies.

In an attempt to maximize vaccine efficiency, we designed MoG to be more broadly representative of all PG-I lyssavirus glycoproteins. MoG has 93% similarity to the wild-type big brown bat variant RABV used in the challenge study. The glycoprotein of the CVS-11 strain has 94.7% consensus amino acid similarity to MoG, but only 90% similarity to the big brown bat variant RABV. The higher level of similarity between MoG and the challenge strain, as compared to the CVS-11 G protein, may have resulted in the slightly higher survival of

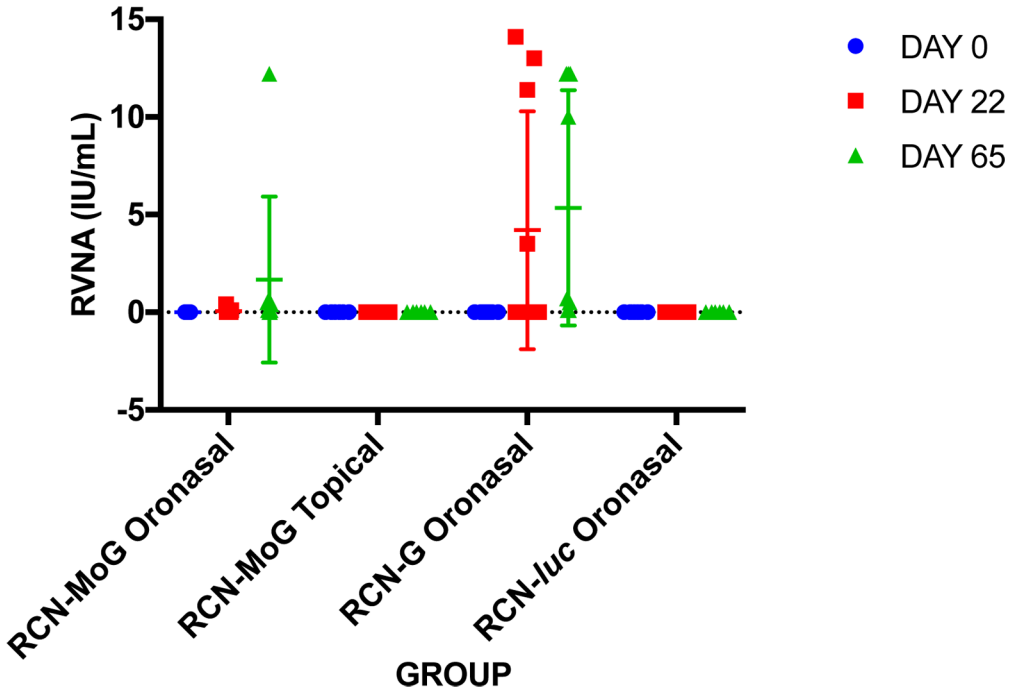

Fig 5. Rabies virus neutralizing antibodies in bats following oronasal vaccination with RCN-based rabies vaccine constructs. Serum rabies neutralizing antibody titers at various time-points as determined by rapid fluorescence focus inhibition test (RFFIT). Day 22 represents levels after initial vaccination, and day 65 represents levels after boost and immediately prior to challenge.

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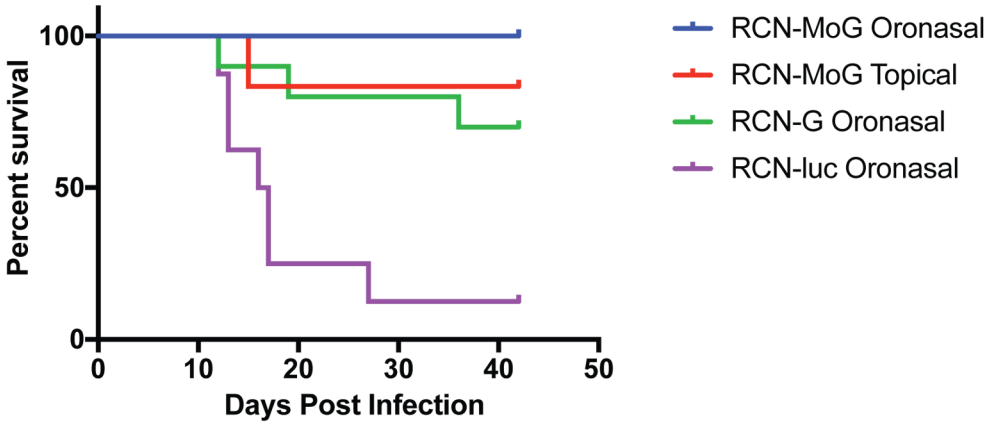

P-values:	RCN-MoG Oronasal	RCN-MoG Topical	RCN-*luc* Oronasal
RCN-G Oronasal	0.08	0.59	0.01
RCN-MoG Oronasal		0.22	<0.01
RCN-MoG Topical			0.02

Fig 6. Survival after rabies challenge in *E. fuscus* bats. Percent survival of *E. fuscus* bats is shown over time after experimental infection. Bats were vaccinated oronasally with RCN-MoG, RCN-G, or RCN-l*uc* (negative control). A fourth group was given RCN-MoG topically in a glycerin jelly vehicle. Vaccinated bats had significantly greater survival than negative controls (P = 0.002).

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RCN-MoG vaccinated bats (survival 9/9) compared to RCN-G vaccinated bats (survival 7/10), although the difference observed between these small groups was not statistically significant.

Mosaic proteins are synthetically designed to represent all potential epitopes from related input sequences and have been shown to induce greater cross-reactivity than consensus sequences [52]. Thus, we expected the immune response elicited by vaccination with MoG to be more efficient at neutralizing naturally circulating RABV than current antigens, however this was not detected by RFFIT (Table 2). Interestingly, RVNA did not correlate directly with survival. Specifically, topically vaccinated bats, as well as some bats vaccinated ON with RCN-MoG, did not seroconvert prior to challenge, yet survived. While it is generally believed that RVNA are needed for protection, results similar to ours have been reported elsewhere [53–56]. In our case, it is possible that the RCN-MoG vaccine may be better at priming T_H cells or activating other adaptive cellular immune responses necessary for clearance of RABV [57–61]. The use of viral vaccine vectors usually leads to a Th1, CTL response directed at the target antigen. The earlier production of antigen due to the S E/L promoter also leads to an increased CTL response[62]. It is possible that CD8 cells, elicited by vaccination with RCN-MoG, lysed infected cells shortly after challenge, resulting in protection in the absence of detectable neutralizing antibody responses. The enhanced inflammatory response induced by activated CD8 T cells may also have contributed to antibody-mediated clearance, as has been previously suggested[60]. In follow-up studies, it would be useful to assess the cellular immune response to vaccination.

Alternatively, it is possible that RVNA induced by RCN-MoG were not properly recognized due to the use of CVS-11 strain in the RFFIT analysis. Thus, it might be necessary to develop a RIFFT assay with MoG as the substrate antigen and to compare the neutralizing capacity of antibodies induced by both RCN-MoG and RCN-G constructs to various divergent lyssaviruses.

The studies presented here are especially relevant for vampire bats. So far, most efforts to reduce their threat have centered on culling through the application of anticoagulants to individual bats that are released to poison additional bats through contact and commensal grooming. Vampire bats in particular are known to practice self and social grooming at a very high rate [63], so this method of application is very effective. Unfortunately, culling of bats has largely failed to reduce the incidence of bovine rabies and may be counterproductive for disease control [49,50,64]. Also, this method frequently leads to indiscriminate killing of other bat species [3], which are key members of their ecosystems. Instead, by immunizing certain vampire bat populations against rabies with sufficient coverage to create herd immunity, it may be possible to reduce rabies transmission, thereby lowering the risk of exposure to humans and livestock.

Previous laboratory studies have demonstrated successful topical vaccination of *Desmodus* using a vaccinia virus expressing the glycoprotein from the ERA strain of rabies (VR-G) [53,65,66]. However, the vaccinia vector can infect humans, especially immunocompromised individuals [18,67], and oral delivery of this vaccine to vampire bats induced lower levels of rabies neutralizing antibodies than oral delivery of RCN-G to *E. fuscus* in this study and *T. brasiliensis* in our previous study [22]. With further testing in vampire bats, RCN-MoG may offer a safer, more effective alternative that could be delivered topically via glycerin jelly or another medium. For a topical vaccine to be practical and effective, it must induce significant immunity after limited oral exposure and must be applied in an appropriate medium that maintains vaccine titer for extended periods in ambient conditions and attaches firmly to the fur of the target species. Although glycerin jelly was effective in our initial studies, more work is required to determine its utility as a delivery medium for free-ranging bats. An alternative to topical

application of vaccine may be aerosolized application to roost sites in caves, but that remains to be tested.

Finally, this approach could be adapted for other species or groups of bats and for other important diseases, such as white nose syndrome, a fungal disease killing millions of bats in North America [68]. While much effort has gone into identifying and characterizing the pathogens carried by bats, little has been done to prevent disease in bat hosts. Successful vaccination of bats against rabies could potentially lead to the development of other bat-targeted vaccines.

Supporting information

S1 Fig. Bat weights over time. Bat weights (in g with SD) are shown over time (each date as a time-point), with vertical bars denoting the date of initial vaccination (1/25), boost dose (3/10), and rabies challenge (3/29). No significant weight loss is appreciable after vaccination with RCN constructs.
(TIFF)

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Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (*Tadarida brasiliensis*)

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Abstract

Bats (Order Chiroptera) are an abundant group of mammals with tremendous ecological value as insectivores and plant dispersers, but their role as reservoirs of zoonotic diseases has received more attention in the last decade. With the goal of managing disease in free-ranging bats, we tested modified vaccinia Ankara (MVA) and raccoon poxvirus (RCN) as potential vaccine vectors in the Brazilian Free-tailed bat (*Tadarida brasiliensis*), using biophotonic *in vivo* imaging and immunogenicity studies. Animals were administered recombinant poxviral vectors expressing the luciferase gene (MVA-*luc*, RCN-*luc*) through oronasal (ON) or intramuscular (IM) routes and subsequently monitored for bioluminescent signal indicative of viral infection. No clinical illness was noted after exposure to any of the vectors, and limited luciferase expression was observed. Higher and longer levels of expression were observed with the RCN-*luc* construct. When given IM, luciferase expression was limited to the site of injection, while ON exposure led to initial expression in the oral cavity, often followed by secondary replication at another location, likely the gastric mucosa or gastric associated lymphatic tissue. Viral DNA was detected in oral swabs up to 7 and 9 days post infection (dpi) for MVA and RCN, respectively. While no live virus was detected in oral swabs from MVA-infected bats, titers up to 3.88×10^4 PFU/ml were recovered from oral swabs of RCN-infected bats. Viral DNA was also detected in fecal samples from two bats inoculated IM with RCN, but no live virus was recovered. Finally, we examined the immunogenicity of a RCN based rabies vaccine (RCN-G) following ON administration. Significant rabies neutralizing antibody titers were detected in the serum of immunized bats using the rapid fluorescence focus inhibition test (RFFIT). These studies highlight the safety and immunogenicity of attenuated poxviruses and their potential use as vaccine vectors in bats.

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Appendix A. Supplementary material: Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.08.088.

1. Introduction

Over the last few decades, the importance of bats (order Chiroptera) in the maintenance and transmission of zoonotic diseases has become increasingly evident; bats are thought to harbor the most zoonotic agents per species [1]. The list of pathogens that infect bats includes the major mammalian paramyxoviruses [2], coronaviruses [3,4], filoviruses [5–7], distinct influenza lineages [8,9], hepadnaviruses [10], and hantaviruses [11], as well as lyssa-viruses such as rabies virus [12,13]. In the United States, bats are often the most common source of rabies infections in humans [14], and in Central and South America, rabies transmitted by vampire bats is a serious zoonotic and economic issue [15]. This association between bats and pathogens that significantly impacts human populations has increased public fear and misunderstanding of these animals and lead to culling campaigns [15–18]. Unfortunately, culling campaigns often lead to the death of valuable non-target bat species [16] and appear ineffective in reducing disease incidence [17]. Alternatively, vaccination of other wildlife species has been successful in mitigating the public health impact of rabies with the development of efficient and practical distribution methods for mass immunization.

Poxviral vectors have been used extensively for oral vaccines to control infectious diseases in a variety of animal species over the last 25 years [19,20]. Several advantages of poxviruses as vaccine vectors include: (1) their allowance of large insertions of foreign DNA; (2) ease of manufacturing; (3) thermal and genetic stability; (4) safety and infectivity for multiple target species; and (5) ability to infect via mucosal and dermal routes. For example, an oral rabies vaccine, constructed by inserting the rabies G glycoprotein into vaccinia virus and distributed via baits, has been used for many years to curtail rabies outbreaks in foxes, raccoons and other animals in North America and Europe [21]. Previous studies assessing a vaccinia-based rabies vaccine (VR-G) in *Desmodus* bats demonstrated the protective efficacy of that construct [22–24]. However, this vector has undesirable side-effects, especially in immunocompromised individuals [25], necessitating the development of attenuated virus strains. More recently, an oral sylvatic plague vaccine using another poxvirus (raccoon pox, RCN) was shown to protect prairie dogs and is currently being tested in large-scale field trials [26,27]. RCN was first isolated from the upper respiratory tract of apparently healthy raccoons in North America [28]. It has since been shown to be safe and effective in a variety of species, including domestic cats, piglets, dogs, raccoons, skunks, foxes, bobcats, rabbits, sheep, prairie dogs, non-human primates, and chickens, with none of the immunized animals showing clinical side effects [29–32]. Additionally, RCN has been shown to be immunogenic via non-parenteral routes in both domestic species [29] and free ranging wildlife [33,34]. Another orthopoxvirus vector, modified vaccinia Ankara (MVA), is a highly attenuated form of vaccinia [35,36] and has also been demonstrated to safely and effectively induce immunity [37,38].

Based on the success of mucosal vaccination with poxvirus vectors in many other species, we hypothesized that poxviruses could be immunogenic and safe when given mucosally in chiropteran species. To test this, we assessed the infectivity and pathogenicity of MVA and RCN in *T. brasiliensis* via *in vivo* imaging studies. The immunogenicity of RCN given

oronasally was also assessed using standard serologic techniques after vaccination with an RCN-based rabies vaccine (RCN-G).

2. Materials and methods

2.1. Ethics statement

The use of bats in this experiment was approved by (Protocol #EP111018) and conducted in accordance with the U.S. Geological Survey (USGS), National Wildlife Health Center (NWHC), Animal Care and Use Committee (ACUC).

2.2. Animals

Adult male bats (*T. brasiliensis; n = 22*) were caught in Brazos County, Texas under Texas Parks and Wildlife Department permit number SPR-1104-610 and Texas A&M University ACUC approval number 2012-130 (courtesy of Mike Smotherman, Texas A&M University, USA). After acclimating to captivity, the bats were transferred to NWHC (Madison, Wisconsin, USA), where all bat studies were conducted under ABSL-3 conditions. Upon transfer to the NWHC, bats were maintained in flight cages for a quarantine period of 30 days. During this time blood samples were taken and bats were treated topically for parasites. Electronic microchip identification units (Avid Identification Systems, Inc., Folsom, Louisiana, USA) were inserted into each animal, between the scapulae, via subcutaneous injection. Bats were maintained on mealworms (*Tenebrio molitor*) supplemented with vitamins and an omega fatty acids mixture, and water was available *ad libitum.* Light cycles were set to 12 h of light per day inverted from the natural cycle to allow monitoring of bat activities during facility working hours.

2.3. Viruses and cells

The RCN-*luc* strain used in this study was previously described [32]. The MVA-GFP strain used to create the MVA-*luc* constructs was generously provided by Inviragen (Madison, WI), while RCN-G [34] was kindly provided by the Centers for Disease Control (Atlanta, GA). Recombinant viruses were generated and amplified on cell monolayers of rat embryonic fibroblasts (Rat-2, ATCC #CRL-1764), baby hamster kidney cells (BHK-21, ATCC #CRL-12072), African Green monkey kidney epithelial cells (Vero, ATCC #CCL-18), or primary chicken embryo fibroblasts (CEF, Charles River Laboratories, INC, Wilmington, WA, USA)). Cell cultures were maintained at 37 °C and 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) or Opti-MEM® (Life technologies, Madison, WI 53719), supplemented with 2–5% fetal bovine serum (FBS). Viruses were titrated prior to use with plaque dilution assays in 6-well plates.

2.4. Construction of recombinant MVA-luc

Recombinant MVA-*luc* viruses were constructed as described elsewhere [39]. Briefly, CEF cells were infected with MVA-GFP at a multiplicity of 0.05 PFU per cell; one hour later the cells were transfected using the FuGENE® reagent protocol (Promega, Fitchburg, WI) with a pI2 transfer plasmid containing (1) DNA flanking segments adjacent to deletion III within the *Hind*III A fragment of MVA, (2) the Red Fluorescent Protein (RFP) under the control of a p11 promoter, and (3) the firefly luciferase gene (*luc*) under the control of a strong

synthetic early/late (SE/L) vaccinia virus promoter upstream Supplementary Fig. S1). At 48-72 h post transfection, the cell cultures were put through three freeze-thaw cycles, harvested, sonicated, and centrifuged at 500g for 5 min at 4 °C. The sonicated cell extracts were plated onto fresh BHK-21 cells and overlaid with 0.8% agarose. After 48–-72 h, through the use of specific microscope filters, the recombinant viruses were detected by the presence of the RFP gene, which replaced the GFP gene during homologous recombination. Selected cell/virus samples were sonicated and plated again as described above. After four consecutive rounds of plaque isolation, recombinant MVA-*luc* virus was confirmed by PCR analysis using OneTaq® Quick-Load® 2X Master Mix with Standard Buffer (New England BioLabs Inc., Ipswich, MA 01938, USA) amplifying the insertion at the Del III flanks using ATGCGGCACCTCTCT-TAA as a forward primer and CCAAAGCTTGCACATACATAAGTA as the reverse primer. The virus subsequently amplified in freshly prepared CEF cells.

2.5. Bioluminescent imaging

Biophotonic luminescent imaging (BLI) has been successfully used to assess the infectivity, infection course, and tissue tropism of viruses and candidate vaccine vectors [40]. Unlike traditional pathogenicity studies that require euthanasia of animals at different time points, BLI allows study of the course of infection over time in a single host, increasing the information gained while reducing the number of animals required.

Groups of 8 wild-caught *T. brasiliensis* were separated into two screened flight cages (33″W × 66″D × 84″H) approximately 5 m apart. Four bats from each group were given 10^9 plaque forming units (PFU) in 100 μl of either RCN-*luc* or MVA-*luc* by intramuscular (IM) injection, split into two 50 μl volumes injected into each thigh muscle. The remaining four bats in each group were given the same amount of virus in 70 μl sterile saline; using a micropipette with sterile tips, the volume split among the nostrils (10 μl given each nare) and mouth (50 μl) for oronasal (ON) exposure. Bats were monitored for 3 h post inoculation for signs of adverse effects. Animals were scanned using an IVIS 200 Biophotonic imager (PerkinElmer, Hopkinton, MA, USA) at one-day post infection (dpi) and every-other day thereafter until 2 consecutive images with less than 100 radiance units (comparable to background) were observed. Bats were scanned prior to infection and confirmed to have no auto-luminescence beyond background levels. Imaging was conducted at roughly the same time period each day, midway through the bats' active period. On imaging days the bats were separated individually into paper lunch bags, weighed, and injected intraperitoneally (i.p) with d-luciferin (Potassium-Luciferin, Gold Biotechnology, St. Louis, MO 63132, dose: 150mg/kg) at 14min prior to imaging the ON group and 26 min prior to imaging the IM group, which were empirically determined to be the time of peak luminescence post substrate exposure on the first day of imaging. All bats were examined for signs of disease or discomfort at the time of substrate injection. For imaging, animals were anesthetized by chamber-delivered isoflurane and positioned in the imager in dorsal recumbency with wings extended laterally, where they were maintained on mask-delivered isoflurane. After imaging the anesthesia was ceased, bats were monitored and given thermal support during recovery. After 11 dpi the bats with remaining detectable luminescence were imaged every 3 days until luminescence was below detectable levels. Images were collected and analyzed using

Living Image software (Caliper Life Sciences, Alameda, California, USA). A region of interest (ROI) was created which covered the entire body of the bat when analyzing the luminescence data.

At 87 dpi, a random group of six bats, all initially exposed to RCN-*luc* by either the ON (n = 4) or IM (n = 2) route, was given a booster exposure to the RCN-*luc* vaccine via the ON route at the same dose. Bats from this group were imaged at 1, 3, and 5 dpi using the same protocol as described above.

2.6. Assessment of viral shedding

During anesthesia for each imaging time-point, oral swabs were collected (CLASSIQSwabs, Copan Flock Technologies, 25125 Brescia, Italy) and placed in a 1.5 ml tube containing 200 μl DMEM media. Fecal samples were also retrieved when available from the bags in which the individuals were contained during imaging, placed in a 1.5 ml tube without media. All samples were quickly stored at -80 °C until testing. Prior to testing, 100 μl DMEM media was added to fecal samples, vortexed thoroughly and sonicated four times in a bath sonicator for 15 s. DNA was extracted from 40 μl of the samples using the Zymo Quick-gDNA™ MiniPrep kit (Zymo Research, Irvine, CA 92614, U.S.A.), and a PCR assay was run to assess for presence of the inoculated virus. PCR was performed with OneTaq® Quick-Load® 2× Master Mix with Standard Buffer (New England BioLabs Inc., Ipswich, MA 01938, USA) and primers targeting the *luc* insert flanks [Supplemental info)]. The limit of detection for this PCR protocol was determined to be 0.125 picograms of DNA, or 6.8×10^4 copies, as determined by serial dilution of a known quantity of viral DNA. Any samples positive by PCR were then assessed for levels of live virus by determining the median tissue culture infective dose ($TCID_{50}$). For RCN-*luc* samples, positive wells were assessed by plaque observation, and the $TCID_{50}$ was calculated and used to approximate plaque forming units (PFU)/ml by the Spearman & Kärber algorithm [41]. For MVA-*luc* samples, positive wells were assessed for viral titer by the luciferase marker using steadylite plus™ (PerkinElmer Inc, Waltham, MA 02451). Serial dilutions from 10^{-2} to 10^{-7} were made, and 100 μl from each was used to infect a 96 well plate with BHK-21 cells at ~80% confluency. Due to minimal sample volume left, no smaller dilutions were possible. After 3 days of infection, 100 μl of the steadylite reagent was added to each well, mixed by pipetting, and after 15 min the plates were scanned in a luminometer (Veritas™ Microplate Luminometer, Turner BioSystems, Inc, Sunnyvale, CA 94085).

2.7. RCN-G immunization

An additional group of five *T. brasiliensis* were housed separately in the same type of caging and given RCN-G via the ON route to assess the immunogenicity of RCN-delivered rabies CVS strain glycoprotein. For this exposure the bats were anesthetized with isoflurane prior to exposure. A dose of 10^8 PFU of viral vaccine was given in 70 μl sterile saline, split between 50 μl orally and 10 μl in each nare. Bats were monitored through their anesthesia recovery for 3 h for potential adverse events. Serum samples were obtained prior to vaccination and at 30 and 60 dpi and tested for the presence of anti-rabies neutralizing antibodies by the rapid fluorescence focus inhibition test (RFFIT). Serum samples from bats given RCN-*luc* (used in the luminescence study) were also collected at 0 and 60 dpi and

used for controls. Serum was collected by making a small lance in the interfemoral vein and collecting up to 200 μl of blood in a capillary tube (Microvette® CB 300 Blood Collection System, Sarstedt AG & Co., Nümbrecht, Germany), which was subsequently centrifuged at 10,000g for 10 min. A micropip-ette was used to collect the serum from the top of the blood container and transfer it to a separate tube for storage at −80°.

Testing was conducted at the CDC Poxvirus and Rabies Branch using standard RFFIT protocols [42], augmented for smaller volumes of serum as previously described [43]. The assay was run in triplicate for each sample, and the results reported represent average titers. Prior to the 60 dpi sample collection, two bats from the RCN-G group were lost from the study due to non-vaccine related mortalities.

2.8. Statistical analysis

Analysis of the data was performed using the R-commander software package [44]. Weight change was analyzed with repeated measures ANOVA, where ‘weight’ is a function of group, route, and time, plus all interactions, using individual bats as the repeated measures. Differences in luminescence were analyzed using a linear mixed-effects model fit by the restricted maximum likelihood approach (REML).

3. Results

3.1. In vivo imaging studies

To assess the infectivity, tissue tropism, and course of infection of RCN and MVA in *T. brasiliensis*, bats were infected with recombinant virus expressing the firefly luciferase gene (*luc*). Two routes were assessed; the IM route and the ON route, which is most biologically relevant for wildlife vaccination. Throughout the study, no clinical signs of disease, lesions, or significant weight loss were observed after administration of viral vectors (Supplementary Fig. S2,Supplementary Table S3). All bats infected with *luc*-expressing poxvirus vectors had detectable expression of luminescence by 1 dpi, and peak levels were observed at 1 and 3 dpi for the IM and ON routes, respectively (Figs. 1 and 2). Viral infection via IM exposure was cleared within 7 days for MVA and 9 days for RCN, while infection after ON exposure was cleared within 9 days for MVA and 21 days for RCN (final images for RCN given ON not shown). Statistical analysis revealed that luminescence was significantly higher ($P = 0.028$) in bats that received RCN compared to MVA and significantly higher ($P = 0.032$) for those administered virus by the IM route compared to the ON route. In the IM injected groups, significant viral spread to other areas was not evident. In contrast, an initial site of viral replication was evident in the oral cavity after ON exposure, often followed by a secondary site of expression further down the gastrointestinal tract. All luminescence measurements are listed in Supplementary Table S4).

In the group of bats re-exposed to RCN-*luc* to assess whether prior exposure would affect the infectivity of the viral vectors, no significant difference ($P = 0.33$) was detected in luminescence when compared to initial exposure through 5 dpi (Fig. 3).

3.2. Assessment of viral shedding

PCR analysis of oral swabs revealed the presence of MVA-*luc* DNA in 5 out of 8 bats up to 7 dpi (3 infected IM, 2 infected ON), however no live virus was recovered. RCN-*luc* DNA was present in 4 out of 8 bats up to 9 dpi (2 infected IM, 2 infected ON), with low levels of live virus detected (6.33×10^3 PFU/ml average, with a median of 9.20 PFU/ml in those with detectable virus). Two bats from the RCN-*luc* group that had live virus detected by oral swabs also had PCR positive fecal samples at 7 and 9 dpi, however no live virus was recoverable from these samples in titration. Viral shedding appears to occur at very low levels independent of route of exposure, and with no evidence of shedding viable MVA.

3.3. Antibody responses to RCN-G

To assess the immunogenicity of RCN, an additional group of bats (n = 5) was infected via the ON route with RCN expressing rabies virus surface glycoprotein (RCN-G). Again, there was no evidence of clinical disease in any of the vaccinated bats in this experiment. The rabies virus G is very well characterized and known to induce protective humoral immunity to rabies virus [30,45,46]. All bats assessed by RFFIT had negligible Ab titers prior to vaccination (Supplementary Table S5). As a control, bats vaccinated with *luc*-expressing virus for the imaging study (N = 7) were bled at 0 and 60 dpi and assessed by RFFIT as well. By 30 dpi all rabies vaccinated bats (5/5) developed Ab titers greater than 0.2 IU/ml (0.20–11.46, with a mean of 5.14), while 7/7 bats that received RCN-*luc* had titers 60.09 IU/ml (Supplementary Table S5). While there is no “protective” level of rabies virus neutralizing antibodies (RVNA), there is a positive correlation between RVNA titers and the level of protection after virus challenge [22,47–50]. Titers between 0.1 and 3.0 IU have been protective for other mammalian species [22,47–50].

4. Discussion

Despite the association between bats and zoonotic diseases, there is currently no significant effort to decrease the incidence of important infectious diseases in these animals. Instead, efforts are more focused on culling of populations (e.g. vampire bats in Latin America) or controlling disease after spill-over into other animal hosts. The continued spillover of rabies virus strains to humans and domestic animals from terrestrial carnivores has led to the development of successful oral rabies vaccination (ORV) programs in Europe and North America. These campaigns often utilize recombinant viral vectors that stimulate immunity to the surface glycoprotein of rabies when ingested orally. When distributed in baits targeted toward certain rabies-carrying species, these vaccines lead to protection from the virus and reduction in local incidence of disease, and even local eradication of some strains of rabies [20,51]. ORV programs are continually evolving and making use of the best available vaccine technology and disease modeling studies. In this study we assessed whether two attenuated pox-viruses might be viable vaccine vectors in a bat species.

Through in vivo imaging studies we have demonstrated that attenuated MVA and RCN are able to infect *T. brasiliensis* via the ON route for a limited time, without causing disease. Our studies demonstrate the tissue tropism and course of infection after exposure of a new animal model, *T. brasiliensis*, to attenuated orthopox-viruses. We show evidence of limited

autologous spread of the viruses after ON exposure, although there is no evidence that the virus spreads outside of GI-associated tissues. MVA was cleared faster and resulted in less detectable luminescence compared to RCN, which may be expected due to the highly attenuated nature of MVA. The lack of significant difference in the levels of viral encoded protein (luciferase) production upon re-infection with RCN, as shown in the booster study (Fig. 3), may be important if bats had been previously exposed to RCN, or if boost inoculations of RCN-vectored vaccine are necessary. While neither vector caused clinical illness, the fact that RCN produced more viral encoded protein (luciferase) over a longer period suggests it is a more immunogenic vector when expressing heterologous antigens. Due to limitations of this study, we were not able to compare the immunogenicity of the vectors directly.

The development of significant levels of rabies neutralizing antibodies after ON exposure to RCN-G demonstrates that the vaccine is highly immunogenic in *T. brasiliensis*. While previous studies have demonstrated the effectiveness of injectable vaccines in this species [52], our study is the first to demonstrate effective mucosal vaccination. An average anti-rabies G titer of 5.14 IU at 60 dpi was detected in bats orally administered RCN-G, which is higher than the levels obtained after vaccination with the VR-G construct in any previous studies, including oral, IM, and scarification routes of exposure (28–30). While these differences may be due to the animal models used, this is additional evidence of the superiority of the RCN vector over vaccinia in bat species. Additionally, the previous studies failed to address the infectivity of vaccinia in the bat host, relying on the lack of clinical disease and development of protective immunity to assess the virus-host interaction. We were unable to assess the duration of immunity past 60 days, but this would be useful information to collect in future studies.

Limited oral shedding of the RCN virus was detected in bats by both PCR and culture of live virus up to 9 dpi, but at very low levels. While there was no evidence of shedding of live MVA virus, there was PCR evidence of vector DNA in oral swabs through 7 dpi; detection of live virus may have been limited due to the method used and the necessary lack of lower dilutions resulting from low sample volumes. One of the advantages of *in vivo* imaging studies is that it is not necessary to sacrifice animals to obtain data, and the bats used in this study went on to be used in a different investigation. Because of this, we were unable to assess organ tissue for evidence of pathology during the infection trials. However, the lack of any apparent morbidity in RCN-treated bats, along with RCN's natural history, record of success in various domestic and non-domestic species, and ability to induce immunity via the oral route, make it a very attractive candidate for use in free-ranging bats.

The results of our experiments provide proof-of-principle that oral vaccination is possible in free-ranging bats. In future work, topical vehicles will be developed that could be used to deliver oral vaccines to the fur coat of bats as they roost or are otherwise congregated. Bats are fastidious animals and spend a large proportion of their time self-grooming [53], which could lead to significant oral exposure to topically applied vaccines. A vaccine that would broadly protect free-ranging bats from rabies virus infection may reduce local incidence of rabies in certain bat populations, limiting the amount of spill-over into humans and other species. *T. brasiliensis* represents a species that roosts in dense colonies and is exposed to

significant levels of circulating rabies virus in their population that may result in human exposures [54]. Therefore, a topically delivered rabies vaccine may be directly applicable to this species, but only if a method for mass application (such as a spray) could be developed. Further research is required to assess the viral vector in other species, such as vampire bats (*Desmodus* spp.), for which topically distributed poison protocols are well developed for culling of colonies [15,55]. While culling has been successful in reducing overall numbers of *Desmodus* at a local level, it has not reduced the incidence of rabies in bat populations and may indeed be counterproductive [17]. Development of topical poxvirus vectored vaccines could potentially lead to effective, applicable, and practical means for reducing disease burden in some free ranging bat populations.

Supplementary Material

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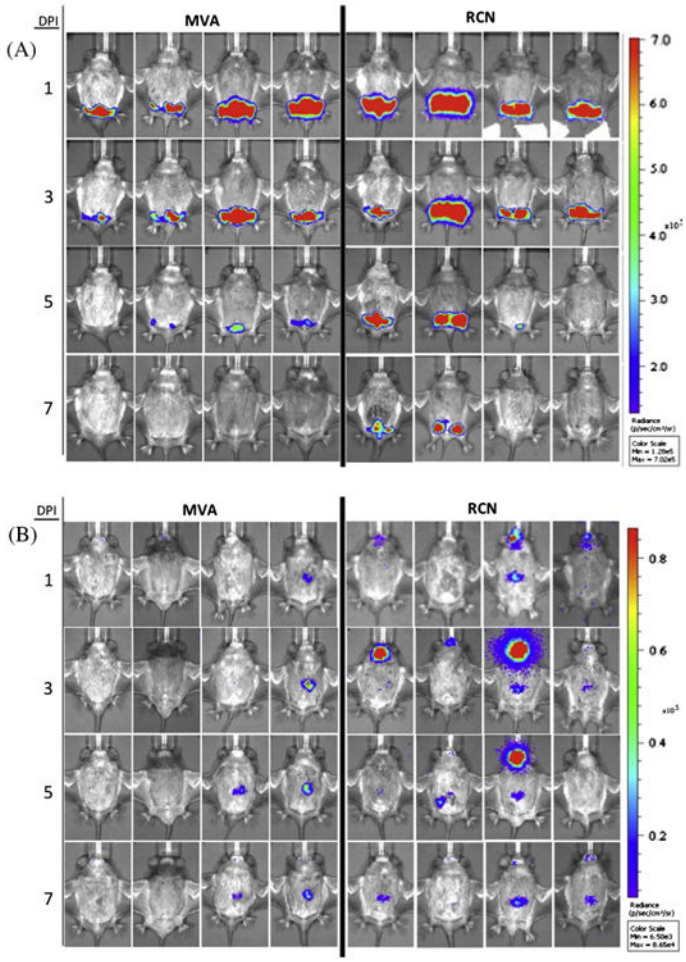

Fig. 1.

Luminescent images for bats given attenuated poxviral vectors, raccoon poxvirus (RCN) or modified vaccinia Ankara (MVA) via intramuscular (IM) (A) or oronasal (ON) routes (B). Images were taken with the IVIS 200 Biophotonic imager and analyzed using the Living Image software. For each vector group, the scale of luminescence is given in photons/second/cm^2/steridian (p/s/cm^2/sr) which has been standardized to compare individuals over time in days post infection (DPI).

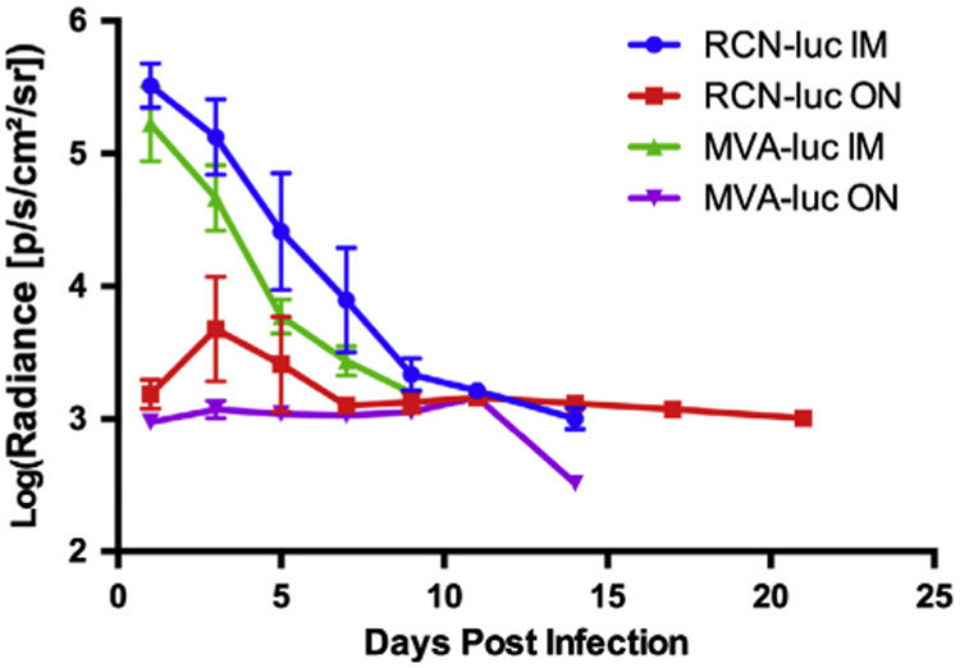

Fig. 2.

Average luciferase expression per vector by route over time for the four groups of bats given luciferase-expressing constructs. Luminescence is given in photons/second/cm^2/steridian ($p/s/cm^2/sr$). Luminescence was significantly higher ($P = 0.028$) for those receiving raccoon poxvirus (RCN) than modified vaccinia Ankara (MVA) and also higher ($P = 0.032$) for those administered virus by the intramuscular (IM) route compared to the oronasal (ON) route.

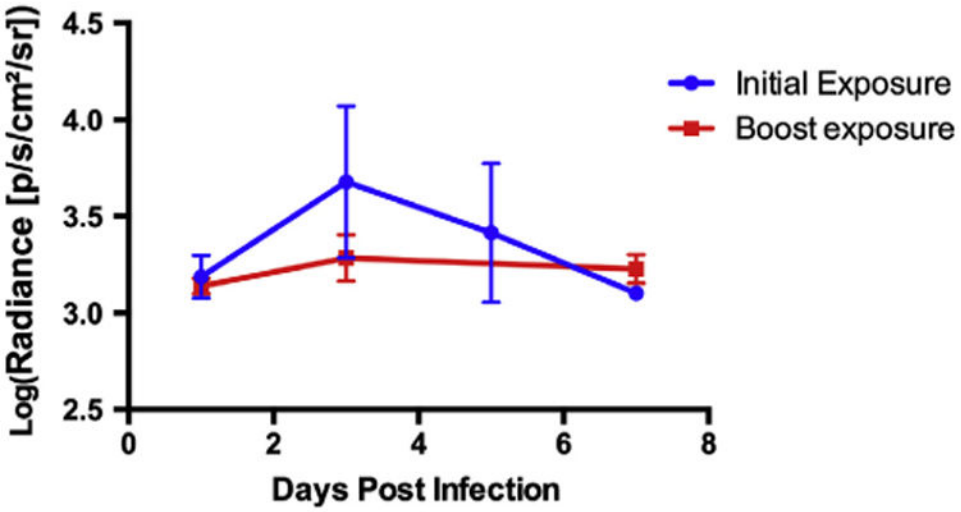

Fig. 3.

Average luciferase expression after oronasal (ON) exposure to raccoon poxvirus (RCN) initially and after re-exposure. Luminescence is given in photons/ second/cm^2/steridian (p/s/cm^2/sr). No significant difference (P = 0.33) was detected in luminescence when compared to initial exposure through 5 days post infection.