Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 05/05/2017

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI110964-03 REVISED
FAIN: R01AI110964

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Understanding the Risk of Bat Coronavirus Emergence

Aleksei Chmura
President
460 West 34th Street
17th Floor
New York, NY 100012317

Award e-mailed to: (b) (6)

Period Of Performance:
Budget Period: 06/01/2016 – 05/31/2017
Project Period: 06/01/2014 – 05/31/2019

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to ECOHEALTH ALLIANCE, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI110964. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Philip E. Smith
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI110964-03 REVISED

Award Calculation (U.S. Dollars)

Salaries and Wages	$167,708
Fringe Benefits	$54,168
Personnel Costs (Subtotal)	$221,876
Materials & Supplies	$7,250
Travel	$35,918
Other	$11,050
Subawards/Consortium/Contractual Costs	$213,239
Federal Direct Costs	$489,333
Federal F&A Costs	$121,757
Approved Budget	$611,090
Total Amount of Federal Funds Obligated (Federal Share)	$611,090
TOTAL FEDERAL AWARD AMOUNT	$611,090
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
3	$611,090	$611,090
4	$597,112	$597,112
5	$581,646	$581,646

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1311726494A1
Document Number:	RAI110964A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2016

IC	CAN	2016	2017	2018
AI	8472350	$611,090	$597,112	$581,646

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C / **OC**: 414E / **Released**: (b) (6) 05/05/2017
Award Processed: 05/05/2017 07:00:56 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI110964-03 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI110964-03 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants

Policy Statement, including addenda in effect as of the beginning date of the budget period.

e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI110964. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

The Research Performance Progress Report (RPPR), Section G.9 (Foreign component), includes reporting requirements for all research performed outside of the United States. Research conducted at the following site(s) must be reported in your RPPR:

San Pya Clinic, BURMA
Institut Pasteur du Cambodge, CAMBODIA
Primate Research Center at Bogor Agricultural University, INDONESIA
Conservation Medicine, Ltd, MALAYSIA
King Chulalongkorn Memorial Hospital, THAILAND
Hanoi Agricultural University, VIETNAM

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REVISED AWARD: This Notice of Award is revised to provide approval for collaboration with the **Wuhan University School of Public Health (CHINA)** in accordance with the request submitted by Aleksei Chmura, Ecohealth Alliance, Inc. on October 6, 2016.

Supersedes previous Notice of Award dated **7/26/2016**.

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REVISED AWARD: This Notice of Award is revised to provide approval for collaboration with the **Wuhan University School of Public Health (CHINA)** in accordance with the request submitted by Aleksei Chmura, Ecohealth Alliance, Inc. on October 6, 2016.

Supersedes previous Notice of Award dated **7/26/2016**.

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No funds are provided and no funds can be used to support gain-of-function research covered under the October 17, 2014 White House Announcement (NIH Guide Notice NOT-OD-15-011).

Per the letter dated July 7, 2016 to Mr. Aleksei Chmura at EcoHealth Alliance, should any of the MERS-like or SARS-like chimeras generated under this grant show evidence of enhanced virus growth greater than 1 log over the parental backbone strain you must stop all experiments with these viruses and provide the NIAID Program Officer and Grants Management Specialist, and Wuhan Institute of Virology Institutional Biosafety Committee with the relevant data and information related to these unanticipated outcomes.

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This Notice of Award (NoA) includes funds for consortium activity with:

- **Wuhan Institute of Virology - CHINA** awarded in the Total Costs amount of **$159,122** (**$147,335** Direct Costs + **$11,787** F&A Costs). Future year commitments are as follows: Year 4 Total Costs: $159,122 and Year 5 Total Costs: $159,122
- **East China Normal University - CHINA** awarded in the Total Costs amount of **$54,117** (**$50,108** Direct Costs + **$4,009** F&A Costs). Future year commitments are as follows: Year 4 Total Costs: $42,300 and Year 5 Total Costs: $32,454

Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps_2013/nihgps_ch15.htm#_Toc271265264.

The written agreement with the consortium must address the negotiated arrangements for meeting the scientific, administrative, financial and reporting requirements for this grant.

No foreign performance site may be added to this project without prior approval of the National Institute of Allergy and Infectious Diseases.

Although a specific amount has been awarded for each consortium, the grantee retains standard rebudgeting authorities.

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This award may include collaborations with and/or between foreign organizations. Please be advised that short term travel visa expenses are an allowable expense on this grant, if justified as critical and necessary for the conduct of the project.

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Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These

individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Jenny L. Greer
Email: (b) (6) **Phone**: (b) (6) **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: (b) (6) **Phone**: (b) (6)

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI110964-03 REVISED

INSTITUTION: ECOHEALTH ALLIANCE, INC.

Budget	Year 3	Year 4	Year 5
Salaries and Wages	$167,708	$167,708	$167,708
Fringe Benefits	$54,168	$54,168	$54,168
Personnel Costs (Subtotal)	$221,876	$221,876	$221,876
Materials & Supplies	$7,250	$7,000	$3,500
Travel	$35,918	$35,918	$35,918
Other	$11,050	$9,800	$9,400
Subawards/Consortium/Contractual Costs	$213,239	$201,422	$191,576
TOTAL FEDERAL DC	$489,333	$476,016	$462,270
TOTAL FEDERAL F&A	$121,757	$121,096	$119,376
TOTAL COST	$611,090	$597,112	$581,646

Facilities and Administrative Costs	Year 3	Year 4	Year 5
F&A Cost Rate 1	44.1%	44.1%	44.1%
F&A Cost Base 1	$276,094	$274,594	$270,694
F&A Costs 1	$121,757	$121,096	$119,376

A. COVER PAGE

Project Title: Understanding the Risk of Bat Coronavirus Emergence	
Grant Number: 5R01AI110964-03	**Project/Grant Period:** 06/01/2014 - 05/31/2019
Reporting Period: 06/01/2015 - 05/31/2016	**Requested Budget Period:** 06/01/2016 - 05/31/2017
Report Term Frequency: Annual	**Date Submitted:** 05/13/2016
Program Director/Principal Investigator Information: PETER DASZAK , BS PHD **Phone number:** (b) (6) **Email:** (b) (6)	**Recipient Organization:** ECOHEALTH ALLIANCE, INC. ECOHEALTH ALLIANCE, INC. 460 W 34TH ST 17TH FLOOR NEW YORK, NY 100012320 **DUNS:** 077090066 **EIN:** 1311726494A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 **Phone number:** (b) (6) **Email:** (b) (6)	**Signing Official:** ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 **Phone number:** (b) (6) **Email:** (b) (6)
Human Subjects: Yes HS Exempt: No Exemption Number: Phase III Clinical Trial:	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then hundreds of novel bat-CoVs have been discovered (including >260 by our group). These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a largescale human-wildlife interface, and high risk of future emergence of novel CoVs.
To understand the risk of zoonotic CoV emergence, we propose to examine 1) the transmission dynamics of bat-CoVs across the human-wildlife interface, and 2) how this process is affected by CoV evolutionary potential, and how it might force CoV evolution. We will assess the nature and frequency of contact among animals and people in two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In the markets we hypothesize that viral emergence may be accelerated by heightened mixing of host species leading to viral evolution, and high potential for contact with humans. In this study, we propose three specific aims and will screen free ranging and captive bats in China for known and novel coronaviruses; screen people who have high occupational exposure to bats and other wildlife; and examine the genetics and receptor binding properties of novel bat-CoVs we have already identified and those we will discover. We will then use ecological and evolutionary analyses and predictive mathematical models to examine the risk of future bat-CoV spillover to humans. This work will follow 3 specific aims:

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. We will examine if: 1) wildlife markets in China provide enhanced capacity for bat-CoVs to infect other hosts, either via evolutionary adaptation or recombination; 2) the import of animals from throughout Southeast Asia introduces a higher genetic diversity of mammalian CoVs in market systems compared to within intact ecosystems of China and Southeast Asia; We will interview people about the nature and frequency of contact with bats and other wildlife; collect blood samples from people highly exposed to wildlife; and collect a full range of clinical samples from bats and other mammals in the wild and in wetmarkets; and screen these for CoVs using serological and molecular assays.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk. We propose two competing hypotheses: 1) CoV host-range in bats and other mammals is limited by the
phylogenetic relatedness of bats and evolutionary conservation of CoV receptors; 2) CoV host-range is limited by geographic and ecological opportunity for contact between species so that the wildlife trade disrupts the 'natural' co-phylogeny, facilitates spillover and promotes viral evolution. We will develop CoV phylogenies from sequence data collected previously by our group, and in the proposed study, as well as from Genbank. We will examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes. We will predict host-range in unsampled species using a generalizable model of host and viral ecological and phylogenetic traits to explain patterns of viral sharing between species. We will test for positive selection in market vs. wild-sampled viruses, and use data to parameterize mathematical models that predict CoV evolutionary and transmission dynamics. We will then examine scenarios of how CoVs with different transmissibility would likely emerge in wildlife markets.

Specific Aim 3: Testing predictions of CoV inter-species transmission. We will test our models of host range (i.e. emergence potential) experimentally using reverse genetics, pseudovirus and receptor binding assays, and virus infection experiments in cell culture and humanized mice. With bat-CoVs that we've isolated or sequenced, and using live virus or pseudovirus infection in cells of different origin or expressing different receptor molecules, we will assess potential for each isolated virus and those with receptor binding site sequence, to spill over. We will do this by sequencing the spike (or other receptor binding/fusion) protein genes from all our bat-CoVs, creating mutants to identify how significantly each would need to evolve to use ACE2, CD26/DPP4 (MERS-CoV receptor) or other potential CoV receptors. We will then use receptor-mutant pseudovirus binding assays, in vitro studies in bat, primate, human and other species' cell lines, and with humanized mice where particularly interesting viruses are identified phylogenetically, or isolated. These tests will provide public health-relevant data, and also iteratively improve our predictive model to better target bat species and CoVs during our field studies to obtain bat-CoV strains of the greatest interest for understanding the mechanisms of cross-species transmission.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Year 2 NIAID CoV Report Final.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Year 2 NIAID CoV Report Professional Development.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

1) Conference and University lectures: PI Daszak, and Co-investigators Shi, Epstein, Olival, Ge, and Zhang gave >100 invited University and Conference lectures including Forum on Microbial Threats (National Academies of Science), Symposium at École du Val-de-Grâce in Paris, Leadership Roundtable at Concordia University Montreal, 1st annual Global Pandemic Policy Summit at Texas A&M Univ., Intl. Conf. of the Wildlife Disease Association in Australia, Intl. Conf. of Conservation Biol in Montpellier France, Michigan State University, Duke University, WDA, ISID conference, Zoological Society of London Symposium, Future Earth meeting, North American Bat Research Symposium, and others that included specific discussion of the current project and results.

2) Agency and other briefings: PI Daszak and Research Technician Dr. Guangjian Zhu introduced this project to potential collaborators within the following agencies: Forestry Dept of Peoples' Republic of China, FAO, TNC, TRAFFIC, China CDC, and TA Foundation in Beijing China in meetings (2015) and also at presentations at the first Wildlife and Public Health Workshop in China (2016) co-hosted by EcoHealth Alliance, the State Forestry Administration of China, and China CDC.

3) Public outreach: PI Daszak presented this work to members of the NIH, NSF, DoD, IUCN, EPA, and the general public, at an EcoHealth Alliance meeting hosted by the Cosmos Club, Washington D.C. (2015); PI Daszak and Co-investigator Zhu reported on this project at a Wildlife Trade and Public Health Seminar, Beijing (2016); PI Daszak introduced this project in a lecture on Pandemics at a New York Academy of Science Panel (2016); Co-PI Y-Z Zhang presented project and results-to-date to department heads and senior researchers at Infectious Disease Departments of four Yunnan Hospitals (2015)

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces.

- Given the reduced amount of wildlife in the local markets within Southern China, and the continued expansion of the Chinese wildlife trade within SE Asia, we would like to conduct short field trips to assess markets, identify wildlife in them, and sample species of bats and other high-risk hosts in countries that neighbor China (Myanmar, Vietnam, Cambodia, Lao PDR) and others that supply wildlife to the international trade to China (Thailand, Malaysia, Indonesia. EcoHealth Alliance has other activities in these countries which would provide leverage to reduce costs of fieldwork, and samples would be tested in Wuhan, China.

- Following the successful collection of ethnographic interviews and focus groups in Year 2, we will be analyzing the qualitative data collection from Years 1 and 2.

- Finalize and conduct survey collection tool for a network study of wildlife farmers using a questionnaire to characterize and map the wildlife value chain.

- After the success of our pilot studies in Year 2, we will continue targeted (at individuals with high risk of exposure to bats), integrated behavioral and biological survey work in Yunnan and expand to Guangxi and Guangdong provinces.

- We will commence our anonymized, surveillance data collection from acutely ill hospital in-patients who satisfy syndromic eligibility criteria; have complete medical records; non-normative laboratory confirmed diagnostic results; and suspected acute viral infection. Eligibility criteria are: (a) suspected acute viral infection; (b) fever > 38˚C, and (c) presenting symptoms of at least one of the following:
•Encephalitis of unknown origin
•Hemorrhagic fever of unknown origin
•Respiratory disease
oInfluenza-like illness (ILI)
oSevere Acute Respiratory like Illness (SARI)
•Rash
•Diarrhea
Some patients with particular infections such as with HIV, HCV, and HBV, may be excluded from the study on that basis. Hospital surveillance has the advantage of monitoring an acutely ill population. Anonymized, passive hospital surveillance allows for data collection and viral testing from all eligible hospital patients thereby limiting population sample bias and increasing the likelihood of identifying positive cases. The strengths of this approach are enormous: an unbiased patient population; prospectively collected, anonymized patient data; a low resource effort with a high efficiency design; and impactful research potential for both case series and case control studies. We have already secured approval from the Institutional Review Boards of the Wuhan School of Public Health and Hummingbird IRB.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk.
Future steps to optimize the model of role of species diversity in CoV emergence risk will include:

- Test and implement our respondent-driven survey to collect specific data on the diversity, abundance, and turnover of species along the wildlife trade network in south China.

- Model viral mixing across the full range parameters found along the wildlife trade network to identify the trade nodes with highest mixing potential. This will include a network analysis of market facility/site connectivity including wild harvest sites, wildlife farming operations, transit holding facilities, and small and large wildlife markets.

- Phylogeographic study of bat-CoV to better understand the geographic distribution and evolution of bat-CoV genetic diversity in south

China.

- Phylogeographic study of bat host (Rhinolophus) species to assess the connectivity of bat populations and infer their historical movements and demographic history to improve our understanding of CoV transmission among bat populations in southern China. Preliminary sequences data has been generated and will be completed and analyzed.

- Cophylogenetic analyses of bat host and CoV phylogenies to assess frequency of cross-species transmission. Comparison of Alpha- and Beta-CoV cophylogenetic patterns building on Year 2 analyses using published sequences and also including Spike gene and additional sequences obtained in Year 2.

- Test and implement our respondent-driven survey to assess diversity, abundance, and turnover of species along the wildlife trade network.

- Examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes;

- Parameterize mathematical models that predict CoV evolutionary and transmission dynamics
- Continued surveillances of SARS-like CoVs and lineage C betacoronaviruses (MERS-related CoVs) in Southern China;

- Full-length genome sequencing and evolution analysis of SARS-like coronaviruses identified from different bat species and different geographical locations across China;

- Full-length genome sequencing and evolution analysis of Lineage C betacoronaviruses identified from different bat species and different geographical locations across China;

- Full-length genome sequencing and evolution analysis of HKU9-related and HKU10-related bat coronaviruses in China;

Specific Aim 3: Testing predictions of CoV inter-species transmission. The following experiments will be undertaken in Year 2:

- Humanized mice with human ACE2 receptors will be infected with WIV1 and the two rescued chimeric SARS-like coronaviruses to determine the tissue tropism and pathogenicity of bat SL-CoV

- Isolation of novel bat coronaviruses. Live virus or pseudovirus will be used to infect cells of different origin or expressing different receptor molecules. Spillover potential for each isolated virus will be assessed.

- An infectious clone of full-length MERS-CoV will be constructed using reverse genetic method. Using the S sequence of different MERS-related viruses identified from Chinese bats, the chimeric viruses with S gene of bat MERS-related coronaviruses and backbone of the infectious clone of MERS-CoV will be constructed to study the receptor usage and infectivity of bat MERS-related coronavirus.

- Surveillance of infection in human populations by SARS-like CoVs. This work will be performed at locations in Yunnan, Guangxi, and Guangdong provinces, in previously identified areas with human populations of high risk of exposure to bats. PCR and ELISA will be used, respectively, for detection of viral replicase gene and antibodies against the viral nucleocapsid protein.

Year 1 Report: Understanding the Risk of Bat Coronavirus Emergence

Award Number: 1R01AI110964-02

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Section B: Accomplishments

B.1 What are the Major Goals of the Project

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then hundreds of novel bat-CoVs have been discovered (including >260 by our group). These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a largescale human-wildlife interface, and high risk of future emergence of novel CoVs. To understand the risk of zoonotic CoV emergence, we propose to examine 1) the transmission dynamics of bat-CoVs across the human-wildlife interface, and 2) how this process is affected by CoV evolutionary potential, and how it might force CoV evolution. We will assess the nature and frequency of contact among animals and people in two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In the markets we hypothesize that viral emergence may be accelerated by heightened mixing of host species leading to viral evolution, and high potential for contact with humans. In this study, we propose three specific aims and will screen free ranging and captive bats in China for known and novel coronaviruses; screen people who have high occupational exposure to bats and other wildlife; and examine the genetics and receptor binding properties of novel bat-CoVs we have already identified and those we will discover. We will then use ecological and evolutionary analyses and predictive mathematical models to examine the risk of future bat-CoV spillover to humans. This work will follow 3 specific aims:

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. We will examine if: 1) wildlife markets in China provide enhanced capacity for bat-CoVs to infect other hosts, either via evolutionary adaptation or recombination; 2) the import of animals from throughout Southeast Asia introduces a higher genetic diversity of mammalian CoVs in market systems compared to within intact ecosystems of China and Southeast Asia; We will interview people about the nature and frequency of contact with bats and other wildlife; collect blood samples from people highly exposed to wildlife; and collect a full range of clinical samples from bats and other mammals in the wild and in wetmarkets; and screen these for CoVs using serological and molecular assays.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk. We propose two competing hypotheses: 1) CoV host-range in bats and other mammals is limited by the phylogenetic relatedness of bats and evolutionary conservation of CoV receptors; 2) CoV host-range is limited by geographic and ecological opportunity for contact between species so that the wildlife trade disrupts the 'natural' co-phylogeny, facilitates spillover and promotes viral evolution. We will develop CoV phylogenies from sequence data collected previously by our group, and in the proposed study, as well as from Genbank. We will examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes. We will predict host-range in unsampled species using a generalizable model of host and viral ecological and phylogenetic traits to explain patterns of viral sharing between species. We will test for positive selection in market vs. wild-sampled viruses, and use

data to parameterize mathematical models that predict CoV evolutionary and transmission dynamics. We will then examine scenarios of how CoVs with different transmissibility would likely emerge in wildlife markets.

Specific Aim 3: Testing predictions of CoV inter-species transmission. We will test our models of host range (i.e. emergence potential) experimentally using reverse genetics, pseudovirus and receptor binding assays, and virus infection experiments in cell culture and humanized mice. With bat-CoVs that we've isolated or sequenced, and using live virus or pseudovirus infection in cells of different origin or expressing different receptor molecules, we will assess potential for each isolated virus and those with receptor binding site sequence, to spill over. We will do this by sequencing the spike (or other receptor binding/fusion) protein genes from all our bat-CoVs, creating mutants to identify how significantly each would need to evolve to use ACE2, CD26/DPP4 (MERS-CoV receptor) or other potential CoV receptors. We will then use receptor-mutant pseudovirus binding assays, in vitro studies in bat, primate, human and other species' cell lines, and with humanized mice where particularly interesting viruses are identified phylogenetically, or isolated. These tests will provide public health-relevant data, and also iteratively improve our predictive model to better target bat species and CoVs during our field studies to obtain bat-CoV strains of the greatest interest for understanding the mechanisms of cross-species transmission.

B.1a Have the major goals changed since the initial competing award or previous report? No.

B.2 What was accomplished under these goals?

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces

In year 2, we continued and expanded the qualitative research begun at the end of Year 1. In addition, a community based integrated biological behavioral surveillance system was developed and pilot tested to identify specific animal exposure risk factors associated with biological evidence of exposure to SARS-like CoV (i.e., seropositive status).

QUALITATIVE RESEARCH

Targeted, in-depth ethnographic interviews were conducted with 47 individuals (18 women; 29 men) in rural Southern China where wildlife trade routes have been documented. Yunnan, Guangxi and Guangdong provinces were specifically selected for study because they have large wildlife populations, a diversity of wildlife species and numerous live animal markets. Individuals who were 18 years of age or older and who were able to provide informed consent were eligible to participate. Twenty-three (49%) in-depth interviews were conducted in Yunnan province at nine different sites, 24 (51%) in Guangxi province at six different sites. In addition, one focus group was conducted in Guangxi. The study was approved by the Institutional Review Boards of the Wuhan School of Public Health and Hummingbird IRB.

Recruitment sites in each province included forested areas or preserves, wildlife farms, hunting areas, wildlife restaurants, live animal markets, caves where people dwell or collect guano and residential areas/farms near known bat caves or roosts. Participants were recruited primarily through local contacts developed as part of wildlife conservation and health research conducted by team members over the past decade. Contacts including wildlife conservationists and researchers, local government health outreach workers and wildlife farmers facilitated introductions and provided referrals. To achieve a sample with sufficient representation of categories of interest, participants were recruited using

purposive sampling, which provides minimum quotas in terms of sex, age and wildlife exposure setting (e.g., live animal market, forest preserve).

The five core themes that guided the in-depth discussions are: 1) human-animal contact, 2) unusual illness experience and response, 3) socioeconomics and daily living, 4) biosafety and 5) human environments and movement/travel. An ethnographic interview guide was developed with examples of questions that could be asked for each theme. In addition, field based participant-observation was ongoing throughout the study and involved observing and talking informally with people in their own natural setting. Field notes were maintained of these ongoing observations and discussions.

Table 1: Species Observed in Wetmarkets in Guangdong Province from 2015 - 2016

Genus species	Common Name
Prionailurus bengalensis	Leopard Cat
Nyctereutes procyonoides	Raccoon Dog
Sus scrofa	Wild Boar
Lepus sinensis	Chinese Hare
Arctonyx collaris	Hog Badger
Hystrix brachyura	Porcupine
Marmota sp.	Marmot
Rhizomes sinensis	Bamboo Rat
Erinaceus sp.	Hedgehog
Mustela putorius	Ferrets
Muridae	Rat (species unknown)
Myocastor coypus	Nutria
Vulpes sp.	Fox
Mustela sibirica	Siberian weasel
Paguma larvata	Masked Palm Civet
Felis catus	Domestic Cat
Canis lupus familiaris	Domestic Dog
Cervinae	Sambar Deer
Ovis aries	Sheep
Capra sp.	Domestic Goat
Ratus norvegicus	Common Rat

Interviews were conducted between March and June 2105 by 10 trained interviewers, none of whom had social science training. Interviewers conducted between one and 22 interviews; three interviewers conducted two thirds of all interviewers. Interviews lasted between 20 and 60 minutes, and were tape-recorded and transcribed verbatim before they were translated into English. All participants received cooking oil valued at US$10 in appreciation of their time.

The data are currently being coded and an analytic database is being constructed. Initial insights include observations by a number of participants, especially those who are older, that there has been a decrease in wildlife in the surrounding environment. This decrease is attributed to many factors including infrastructure development. The government has invested resources to build new roads and renovate local infrastructure with the intention of increasing tourism. This has reduced forested area.

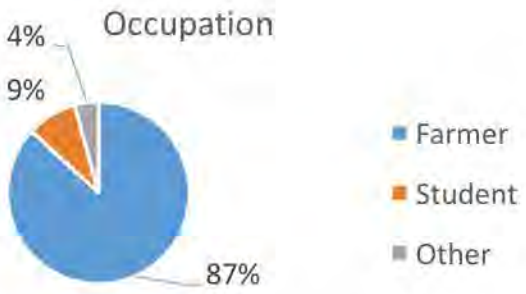
Observations by research staff in live animal markets in Guangzhou found wildlife to be plentiful (see Table 1), although no bats were seen for sale during the observation period. In contrast, wildlife was not found in live animal markets at the sites we visited in either Yunnan or Guangxi. This is a change from previous research visits to the same or similar communities, when bats, rodents and wild boar could be found. Locals in Yunnan and Guangxi attribute the change to conservation law enforcement. The success of conservation enforcement may have moved hunting and trapping underground and made the capture of local wildlife less economically feasible than other income generating activities.

Preliminary analyses are underway. Three specific studies in support of Specific Aim 1 are being developed: the changing wildlife trade in Southern China, the economics of wildlife farming, and zoonotic disease risks resulting from a rapidly changing wildlife trade.

INTEGRATED BIOLOGICAL BEHAVIORAL SURVEILLANCE PILOT STUDY

Currently, mechanisms of zoonotic viral spillover are unknown. In order to evaluate potential risk factors, it is necessary to measure both exposure and outcome data. Therefore, a behavioral risk survey was developed that assessed both animal exposure and experiences of unusual illness both during lifetime and in the past 12 months. In addition, participants were requested to provide serum to test for previous exposure to SARS-like CoV. The integrated surveillance was pilot tested in October 2015 among residents living near bat caves or roosts where SARS-like-CoV has been previously detected in the bat population in Jinning County, Yunnan. Please view the full survey here:

https://www.dropbox.com/s/sv62neywuvl027r/Questionnaire%20Complete.docx?dl=0



Of 218 participants, 139 (64%) were women and 79 (36%) were men, with a mean age of 48 (range: 12-80). Most reported being farmers (87%, and see chart to left); a majority were long term residents (97%). Animal exposures in the past year were extensive, including general (e.g., buying live animals at markets [61%]) and intimate (e.g., being scratched or bitten [9%], slaughter [38%]). In fact, two-thirds of participants reported handling recently killed animal parts and 2 out of 5 reported slaughtering animals. Only 20 (9%) participants reported known exposure to bats.

Standardized syndromic case definitions informed questions concerning unusual illness experience (e.g. severe acute respiratory infections [SARI], influenza-like illness [ILI]). Lifetime, 12 month and unusual illness experience in family for the past 12 months were assessed for all participants. In the past year, SARI was reported by 4 (2%) respondents and for 4 additional family members. Table 2 provides data for all unusual illness experience assessed. None of the participants were found to be seropositive for SARS-like CoV.

Table 2. Unusual Illness Experience

Symptoms	Ever	Past 12 months	Family (12m)
Severe Acute Respiratory Infections (SARI)	15 (6.9%)	4 (1.8%)	4 (1.8%)
Influenza Like Illness (ILI)	54 (24.8%)	16 (7.3%)	26 (11.9%)
Encephalitis	19 (8.7%	4 (1.8%)	3 (1.4%)
Hemorrhagic Fever	0 (0.0%)	0 (0.0%)	0 (0.0%)
Fever with Diarrhea /Vomiting	12 (5.5.%)	2 (0.9%)	3 (1.4%)
Fever with Rash	2 (0.9%)	2 (0.9%)	3 (1.4%)

Although the sample size was small, animal exposures among those who reported unusual illness experiences in the past 12 months were evaluated. Of the four respondents who reported SARI symptoms, 75% reported: raising animals, animals in the home, preparing recently killed animals and buying live animals; 50% reported slaughter. Among the 16 respondents who reported ILI symptoms, 12 (75%) reported handling/preparing recently killed animals, 11 (69%) Handling live animals or having animals in the home, 10 (63%) reported slaughtering/killing animals or buying live animals at wet market, 9 (56%) raised live animals, 7 (44%) reported a pet, and 1 (6%) reported animal feces near food or eating animal touched or damaged food, hunting, or eating raw/undercooked animal products. Finally, among the four respondents who reported encephalitis symptoms, 3 (75%) reported hunting, handling or raising animals, 2 (50%) reported animals in the home, 1 (25%) reported having animals as pets, slaughtering/killing animals, or having bought live animals at wet market.

Respondents were asked about the source of their unusual illnesses. None reported any kind of animal exposure as a potential source of infection and most stated they had no idea how they had become infected. However, when asked about potential behavior changes made at live animal markets in the last 12 months, participants reported a great deal of change. In particular, respondents reported buying live animals less often (38%), only buying farmed wildlife (54%) or buying meat at the supermarket (23%). (See Table 3).

Table 3: Behavior Change at Wet Market in the last 12 months

Behavior	N	(%)
Wear a mask	4	(3.0)
Wear gloves	5	(3.8)
Wash hands	80	(60.6)
Sometimes shop for meat at supermarket	30	(22.7)
Buy live animals less often	50	(37.9)
Buy only farmed wildlife	71	(53.8)
No longer buy wildlife at wet market	39	(29.5)

The results of this pilot study conducted with a largely female farmer population found high levels of unusual illness, as well as high levels of exposure to animals. There was a notable lack of knowledge of animals' ability to transmit infection. Despite this lack of knowledge, there may be a sense of unease about animal exposures, given the fairly dramatic behavior changes reported at live animal markets. The finding of a reduction in wildlife purchase may be due to sensitivity to the legality of wildlife trade, biasing respondents towards not admitting purchasing wildlife. Although, there were no participants seropositive for SARS-like CoV, serological data may add support to the findings from self-reported syndromic surveillance, once serological assays are optimized.

In preparation for full implementation of the integrated biological behavioral surveillance, the survey has been programmed as an application for use on either a mobile device or computer. Electronic data collection will facilitate survey implementation in the field and quality control of the data being collected. Four field team leads were trained on behavioral survey data collection, data collection technologies (the tablet application) and analysis.

Nucleic acid test results of human biological samples

Testing High-Risk Human Populations for Coronavirus Infection

Surveillance of CoV infections in human populations by SARS-like CoVs was significantly expanded in Year 2, including both custom-built ELISA serology (an assay developed by the Wuhan Institute of Virology to test antibodies against the N protein of SL-CoV) and PCR detection of viral RNA.

Serological test for SL-CoV antibodies in human samples from Jinning, Yunnan Province
In order to assess past exposure to bat CoVs, 223 human sera samples were collected in villages in proximity to the bat habitat from which two SL-CoVs with potential for interspecies infection, WIV1 and WIV16, were discovered in our previous research. An ELISA developed by the Wuhan Institute of Virology was used to test antibodies against the N protein of SL-CoV. A number of human specimens generated high OD values and neutralization test to WIV1 and WIV16 was then performed. These findings are encouraging; however, no neutralization antibodies were detected. In Year 3, we will continue to validate and optimize these ELISA assays and other serological tests to obtain data on past CoV exposure.

PCR test for CoV Nucleic Acid in human samples from several Provinces
We tested 405 individual human samples for CoV RNA to identify evidence of active infection in human populations and to obtain sequence data on strain variation. Individual samples (4 each) were pooled prior to nucleic acid extraction then tested using PCR. When a group tested positive, we then conducted the confirmation test in the individual samples. One single sample (14XN611) from someone who had identified as having had a fever and suffered both a cough and headache in the past 7-days was then identified to be positive for HCoV-HKU1. The low number of PCR detections in human specimens is not unexpected, and will be improved in Year 3-5 by better targeting syndromic individuals for specimen collection and continuing to optimize PCR assays. Refined serological assays (above) will provide sufficient data to assess past exposure to specific CoV lineages, and optimizing of PCR detections will allow for more CoV positive human sequences moving forward.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk

Bat CoV PCR detection and sequencing from live-sampled bat populations
We collected 1,714 anal swab samples, 677 fecal samples, 53 blood samples, and 38 serum samples from 15 bat genera in Guangdong, Yunnan, Sichuan, Hubei, Hunan, Guizhou, Guangxi provinces (Table 4).

Table 4 Bat Samples collected for CoV surveillance in 2015

Sample date	Sample location	Anal	Fecal	Blood	Serum
Mar. 2015	Huidong, Guangdong	69	--	--	--
Jun. 2015	Guangdong	495	--	12	--
Apr. 2015	Menglun, Yunnan	51	--	--	--
May 2015	Jinning, Yunnan	--	193	--	--
May. 2015	Mojiang, Yunnan	93	--	--	--
Oct. 2015	Jinning, Yunnan	30	--	--	--

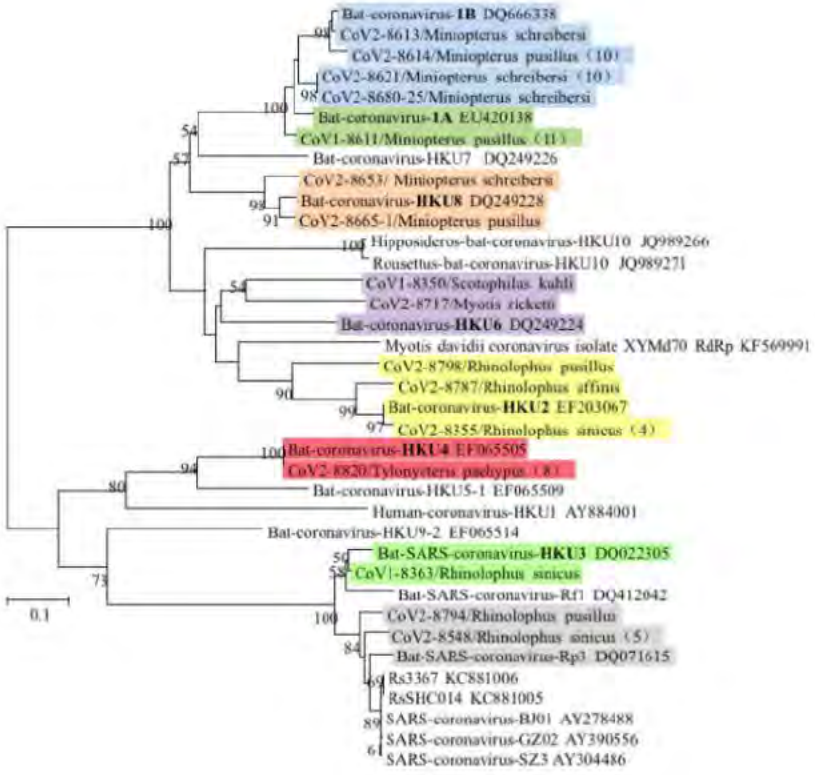
Dec, 2015	Jingna, Yunnan	15	15	13	13
	Miaoxin, Yunnan		42	28	25
Jul, 2015	Zigong, Sichuan	128	--	--	--
Aug, 2015	Hubei		332		
Sep, 2015	Xianning, Hubei		95		
Aug, 2015	Jishou, Hunnan	204			
Aug-Sep, 2015	Tongren, Guizhou	438			
Dec, 2015	Longzhou, Guangxi	191			
	Total	1714	677	53	38

We tested 2,256 samples for CoV RNA and 280 tested positive. The total positive rate is 12.4% (Table 5). Diverse alphacoronaviruses related to Bat CoV 1A, 1B, HKU2, HKU6, HKU7, HKU8 and HKU10 were identified; SARS-like coronaviruses were detected in *Rhinolophus* bats in both Yunnan and Guangdong (Fig 1). Novel lineage B betacoronaviruses more distantly related to SARS-CoV than other SL-CoVs were detected in *Vespertilo superans* in Sichuan. HKU4-related coronaviruses were found in *Tynolycteris pachypus* in Guangdong and Guangxi while HKU5-related coronaviruses were found to be highly prevalent in *Vespertilio superans* in Zigong, Sichuan (41 bats out of 128 tested positive).

Table 5 Test result of bat CoV surveillance in 2015 – 12% positive (280/2,256)

	Yunnan	Guangdong	Hubei	Sichuan	Guangxi	Guizhou	Hunan	Total
Bat species	**No.positive/No.tested**							
Rhinolophus spp.	47/98	12/103				16/225	8/63	83/489
Hipposideros spp.	0/35	0/51	26/152			0/131	0/91	26/460
Ia io						0/3		0/3
Pipistrellus spp.	1/1	0/19				0/2	0/4	1/26
Miniopterus spp.	6/7	34/83				2/6		42/96
Eonycteris spp.	0/3							0/3
Vespertilio superans				41/128				41/128
Myotis spp.		1/38				0/70	0/35	1/143
Taphozous spp.	0/25					0/1		0/26
Tynolycteris pachypus		8/25			27/191			35/216
Scotophilus kuhlii		1/1						1/1
Eptesicus fuscus		0/1						0/1
Tadrida spp.		0/5						0/5
Barbastella							0/1	0/1
Nyclatus velutiaus							0/10	0/10
Fecal samples	28/468		22/180					50/648
Sub-total	82/637	56/326	48/332	41/128	27/191	18/438	8/204	**280/2256**

A



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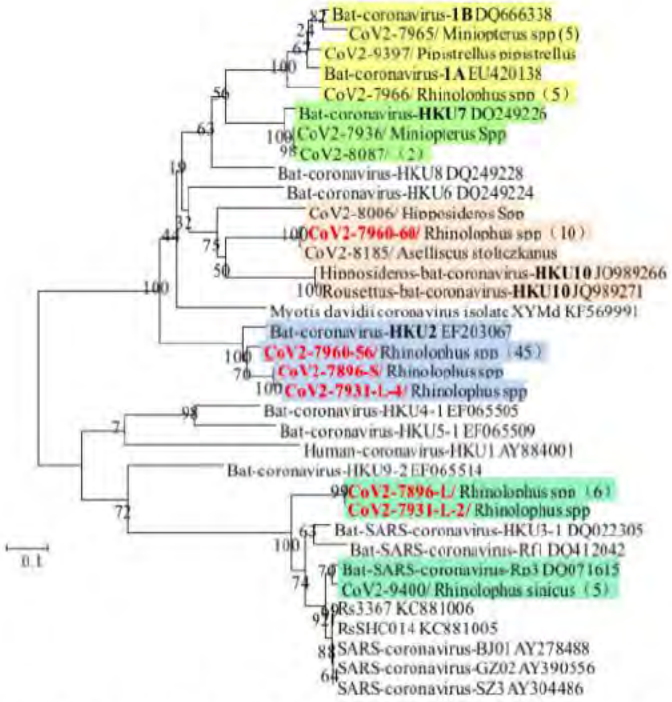


Fig 1 Phylogenetic analysis of partial RdRp gene of CoV (440-nt partial sequence). CoVs identified in 2015 are named by the sample numbers. Sequence amplified from samples co-infected with two CoV strains are indicated in red. (A) CoVs detected in Guangdong. (B) CoVs detected in Yunnan.

Cophylogenetic analysis of CoV host switching

We completed preliminary cophylogenetic analysis of bat host – CoV sequences using data published in the literature and available on Genbank. Two figures from these analyses are highlighted below (Figs 2 and 3) and these methods are currently being extended using partial RdRp CoV and bat mitochondrial DNA sequences from a large number of bat specimens found CoV positive in Year 2 (Table 5, above).

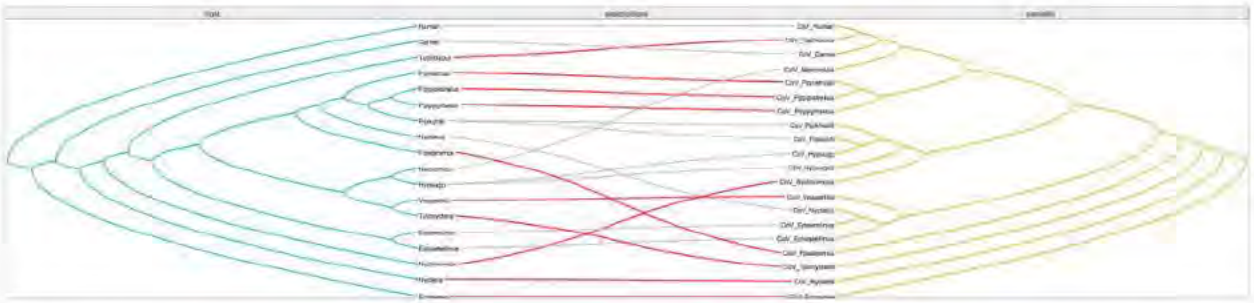


Figure 2: Tanglegram depicting the pattern of infection of bats (and outlier mammalian hosts) by CoVs. The CoV tree was reconstructed from DNA sequences available in GenBank (partial RdRp gene) using Bayesian inference (MrBayes). The topology of host tree was reconstructed using the mammal and bat phylogenies available in Asher & Helgen (2010) and Agnarsson et al. (2011), using methods our group has previously applied to bat parasite cophylogenetic analyses (Lei and Olival 2014). Both ParaFit (ParaFitGlobal = 64957.61, p-value = 0.001) and PACo (m2 = 366.44, p-value = 0.013) provided evidence for significant global congruence between the two topologies, and evidence for coevolution. Lines connecting taxa indicate host-CoV associations. Red lines indicate significant host-CoV associations as indicated by ParaFit ($p \leqslant 0.05$, 999 permutations).

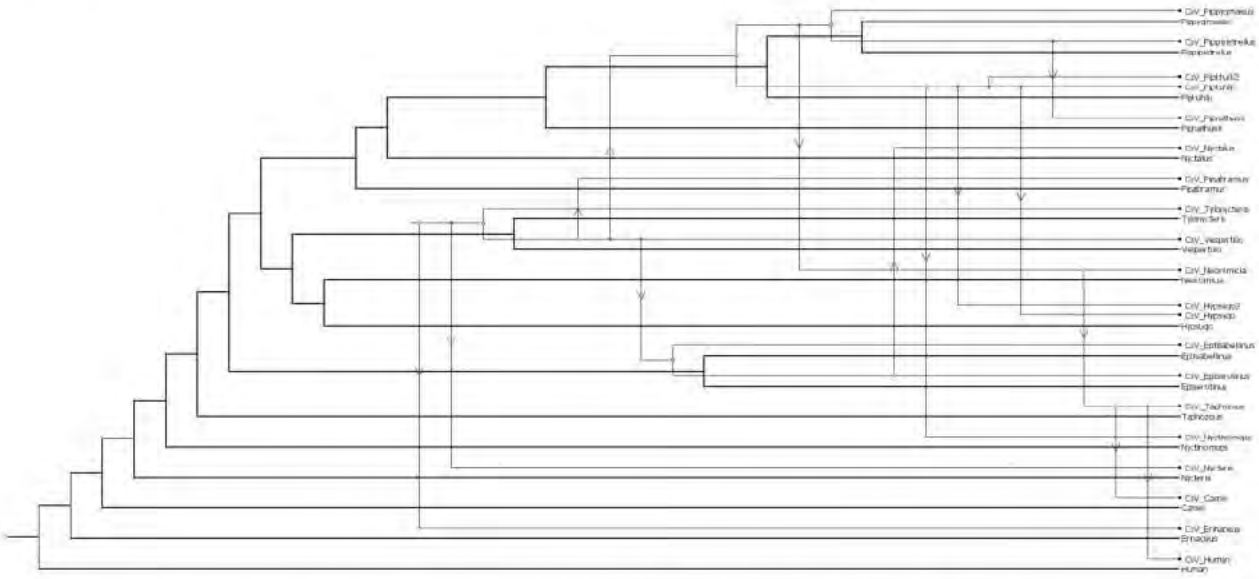


Figure 3: Reconstruction of one of 3 potentially optimal solutions of reconciled host-CoV trees recovered from a Jane analysis. Black and blue lines represent the host and CoV trees, respectively. For each solution, the number of co-speciation events inferred by Jane was always significantly greater than expected by chance. Jane inferred 4 co-speciation events (hollow colored circles), 1 duplication (solid

colored circle), 14 host switches (solid colored circle with arrow), 0 loss and 0 failure to diverge.

Our findings demonstrate co-speciation alone is not sufficient to explain the observed co-phylogenetic pattern and several host switches can be specifically identified. This is the case even if a significant global signal of co-speciation has been detected. This work highlights, the need for these types of detailed cophylgoenetic analyses to best explain the evolutionary history and host-switching of bat-CoVs.

References cited for the above analysis: Agnarsson, I., Zambrana-Torrelio, C.M., Flores-Saldana, N.P. & May-Collado, L.J. (2011) A time-calibrated species-level phylogeny of bats (Chiroptera, Mammalia). *PLOS Currents*, 3:RRN1212. Asher, R.J. & Helgen, K.M. (2010) Nomenclature and placental mammal phylogeny. *BMC Evolutionary Biology*, 10, 1-9. Lei BR, Olival KJ (2014) Contrasting Patterns in Mammal–Bacteria Coevolution: *Bartonella* and *Leptospira* in Bats and Rodents. *PLoS Negl Trop Dis* 8(3): e2738.

Market Characterization Model Parameterization

Our ongoing observational research and mapping of farms and markets suggests that rapid changes in the market and regulatory environment are changing the nature and location of the wildlife market trade. The nexus of the wildlife trade and the potential hotspots of interspecies viral mixing is now in many cases in animal storage facilities and transport between high-volume customers. To define realistic parameters for intermixing wildlife species in areas of high potential mixing, we have developed a preliminary survey and sampling protocol to assess these values as animals move along the value chain – through these storage facilities - using respondent-driven questionnaires to follow and sample along the wildlife trade network and reveal hidden nodes and sites of intermixing of species.

We have expanded our intermixing modeling framework to incorporate the variations along this value chain, where the diversity, abundance, residence time, and contact rates between species change as animals move through the trade network.

Specific Aim 3: Testing predictions of CoV inter-species transmission.

In Year 2, we continued surveillance for novel SARS-like CoVs from bats in Yunnan and Guangdong provinces and obtained full genome sequence for 11 CoV isolates. Full genome analysis of these CoV isolates was completed, including phylogenetic and recombination analyses. Importantly, recombination analysis of the full-length SL-CoV genome sequences from a single bat population revealed that frequent recombination events among different SL-CoV strains occur. Several SL-CoVs that are more genetically similar to SARS-CoV (2003) than any previously discovered were also identified from bat populations in Yunnan province. Full genome analysis suggests that an epicenter of SL-CoV occurs in rhinolophid bats and provides more insight into the evolutionary origin of SARS-CoV.

Full-length genome sequencing of SL-CoVs identified from a single bat colony

To date, including preliminary data submitted for this R01 that we are now analyzing under the current funding, we have conducted 5-years of surveillance of SL-CoV in a single bat colony in Yunnan Province (from 2011 to 2015), leading to the discovery of diverse novel SL-CoVs. Based on genotyping of these SL-CoVs by the region corresponding to the receptor-binding domain (RBD) of SARS-CoVs, 11 isolates were selected and full-length genome sequencing was performed in Year 2.

These SL-CoVs, including four others isolated previously from this colony, Rs3367, RsSHC014, WIV1 and WIV16, are highly diversified in the S gene, but share similar sequence identity to SARS-CoV in ORF1ab (Fig 4). Genomic phylogenetic analysis showed that the SL-CoVs detected in this colony are more closely

related to SARS-CoVs from other geographic regions, especially three isolates, WIV16, Rs4874 and Rs4231 (Fig 5). Notably, among the 15 SL-CoVs, two isolates, Rs4084 from *Rhinolophus sinicus* and Rf4092 from *Rhinolophus ferrumequinum*, are highly similar to SARS-CoV in the ORF8 region (Fig 5). Rf4092 possessed a single ORF8 of the same length (369bp) as that in civet SARS-CoV SZ3, and the sequence showed only 10 nucleotide substitution (Fig 6). The ORF8 sequence of Rs4084 is highly similar to that of Rf4092, however in the region corresponding to the 29-bp deletion acquired in human SARS CoVs (e.g Tor2), a shorter deletion of only 5-bp is present, resulting in two overlapping ORF8s, ORF8a and ORF8b. The position of start codon and stop codon of the two ORFs were consistent with those in human strains (Fig 6).

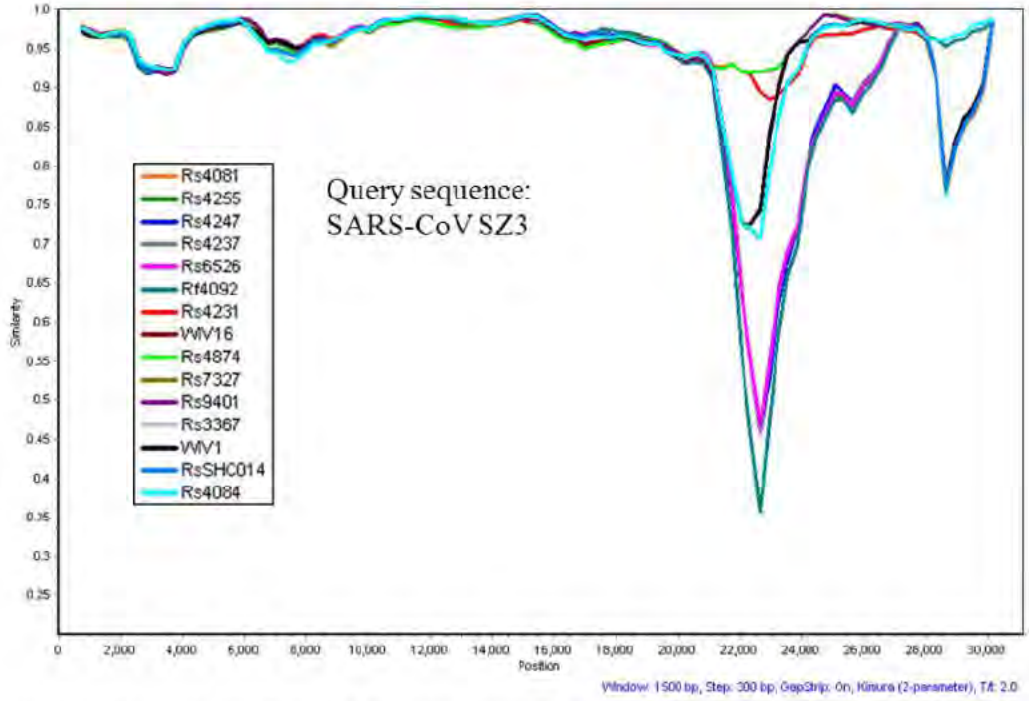


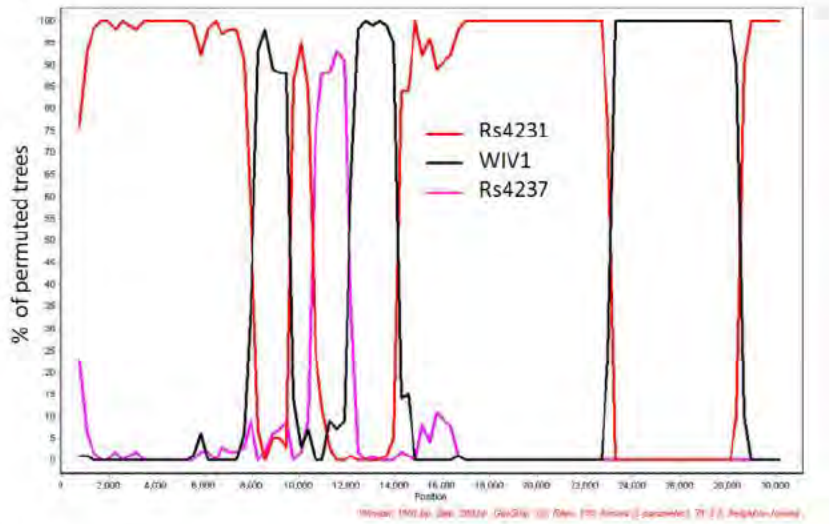
Fig 4. Simplot analysis of the 15 SL-CoVs identified from a single bat colony in Yunnan. SARS-CoV SZ3 is used as query sequence.

Fig 5. Phylogenetic analysis of full-length genome sequences of SL-CoVs and SARS-CoVs. Isolates identified in the single investigated bat colony in Yunnan in in bold.

Fig 6. Alignment of ORF8 nucleotide sequences of SARS-CoV and bat SL-CoVs. The red box indicates the 29-nt deletion present in SARS-CoV of middle and late phase.

Recombination analysis of the full-length genome sequences reveals frequent recombination events among different SL-CoV strains circulating in this bat population. For example, WIV16 appears to be a recombination product of WIV1 and Rs4231. An important breakpoint is identified between the N-terminal domain (NTD) and RBD region in the S gene (Fig 7A). Consequently, WIV16 is identical to Rs4231 and WIV1 in NTD and RBD of the spike protein, respectively, and is highly homologous to SARS-CoV in both NTD and RBD. This makes it the SL-CoV most closely related to the direct progenitor of SARS-CoV discovered to date. Moreover, evidence is found to support the hypothesis that the direct progenitor of SARS-CoV was generated from recombination of WIV16 with Rf4092 at the site near ORF8. This work, which identifies diverse SL-CoVs highly homologous to SARS-CoV in different regions of the genome, suggests that rhinolophid bats are an evolutionary epicenter of SL-CoV and offers more insights into the evolutionary origin of SARS-CoV.

A.



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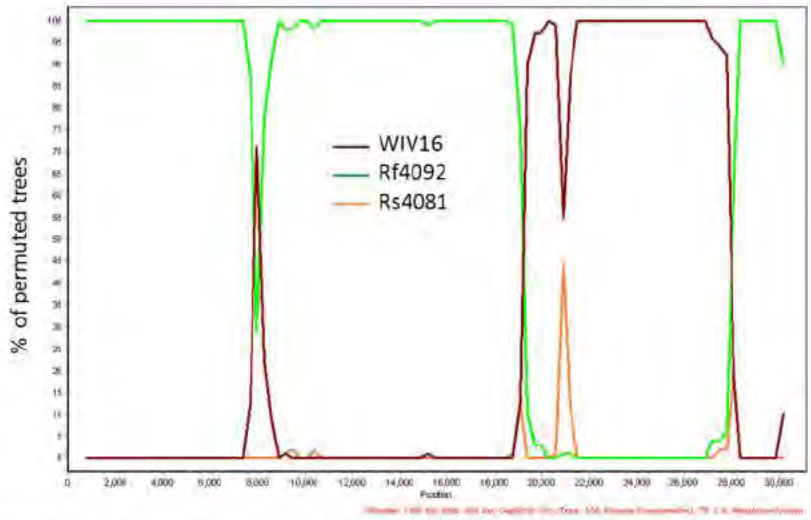

Fig 7 Bootscan analysis of full-length genome sequences of SL-CoVs. (A) WIV16 is used as query sequence. (B) SARS-CoV SZ3 is used as the query sequence. (Kimura model, window size, 1500bp, step size, 300bp)

Additional Year 2 items for Specific Aim 3:

- The infectious clone of WIV1 was successfully constructed using reverse genetic methods;
- Two chimeric bat SARS-like coronavirus strains were constructed by replacing the S gene in the backbone of WIV1;
- Permission to import mice with human ACE2 to China was obtained, so as to conduct the experimental infections proposed in our R01 specific aims.

Specific Goals Not Met.

- Comparative cophylogenetic analyses of bat host and CoV RdRp and Spike gene phylogenies, to assess patterns of evolutionary congruence and frequency of cross-species transmission (This will be conducted in year 3);
- Animal infection experiments of SARS-like coronaviruses were not done, because of the unavailability of mice with human ACE2 in Year 2. We now have secured these mice and will begin this work in year 3.
- Sampling of bat and other mammalian species in markets to screen for CoVs. We will begin this work in year 3.

Section C: Accomplishments: Publications

PUBLISHED

Xing-Yi Ge, Ning Wang, Wei Zhang, Ben Hu, Bei Li, Yun-Zhi Zhang, Ji-Hua Zhou, Chu-Ming Luo, Xing-Lou Yang, Li-Jun Wu, Bo Wang, Yun Zhang, Zong-Xiao Li, and Zheng-Li Shi. Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft. *Virologica Sinica* 31, 31–40 (2016).

Mei-Niang Wang, Wei Zhang, Yu-Tao Gao, Ben Hu, Xing-Yi Ge, Xing-Lou Yang, Yun-Zhi Zhang, Zheng-Li Shi. Longitudinal surveillance of SARS-like coronaviruses in bats by quantitative real-time PCR, *Virologica Sinica* 31(1): 78-80 (2016).

Cristin C. W. Young and Kevin J. Olival. Optimizing Viral Discovery in Bats. *PLoS ONE* 11(2) (2016).

Kevin J. Olival. To Cull, or Not To Cull, Bat is the Question. *Ecohealth* 13, 6–8 (2015).

Xing-Lou Yang, Ben Hu, Bo Wang, Mei-Niang Wang, Qian Zhang, Wei Zhang, Li-Jun Wu, Xing-Yi Ge, Yun-Zhi Zhang, Peter Daszak, Lin-Fa Wang, Zheng-Li Shi. Isolation and characterization of a novel bat coronavirus closely related to the direct progenitor of Severe Acute Respiratory Syndrome Coronavirus, *Journal of Virology* 90(6): 3253-6 (2015).

Ben Hu, Xingyi Ge, Lin-Fa Wang, Zhengli Shi. Bat origin of human coronaviruses. *Virology Journal* 12 (1): 221 (2015)

ACCEPTED, IN PRESS

Lei-Ping Zeng, Yu-Tao Gao, Xying-Yi Ge, Qian Zhang, Cheng Peng, Xinglou Yang, Bin Tan, Jing Chen, Aleksei Chmura, Peter Daszak, and Zheng-Li Shi. Bat SARS-like coronavirus WIV1 encodes an extra accessory protein ORFX involving in modulation of host immune response. *Journal of Virology* (in press, 2016)

B.4 What opportunities for training and professional development has the project provided?

We presented our project to graduate students, laboratory personnel, directors, and doctors from three Hospitals in Yunnan Province: Yunnan Provincial Institute of Endemic Diseases Control & Prevention (YNCDC); Dali Provincial Hospital; and The Third People's Hospital of Kunming. Select doctors at YNCDC (1) and Dali Provincial Hospital (3) were trained in the passive Hospital surveillance project protocols.

We trained graduate students from Dali School of Public Health (1) and the Wuhan University School of Public Health (3) in qualitative behavioral risk data collection methodologies and data collection technologies, survey data collection and analysis. These were also enrolled in and passed the Human Subjects Research Course provided by the Collaborative Institutional Training Initiative (CITI Program) at the University of Miami (http://citiprogram.org). The CITI Program is a leading provider of research education content with web based training materials serving millions of learners at academic institutions, government agencies, and commercial organizations in the U.S. and around the world.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Yang XL, Hu B, Wang B, Wang MN, Zhang Q, Zhang W, Wu LJ, Ge XY, Zhang YZ, Daszak P, Wang LF, Shi ZL. Isolation and Characterization of a Novel Bat Coronavirus Closely Related to the Direct Progenitor of Severe Acute Respiratory Syndrome Coronavirus. J Virol. 2015 Dec 30;90(6):3253-6. PubMed PMID: 26719272; PubMed Central PMCID: PMC4810638.
Complete	Olival KJ. To Cull, or Not To Cull, Bat is the Question. Ecohealth. 2016 Mar;13(1):6-8. PubMed PMID: 26631385; PubMed Central PMCID: PMC4833651.

Non-compliant Publications Previously Reported for this Project

Public Access Compliance	Citation
Non-Compliant	(b) (4)

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
(b) (6)	Y	DASZAK, PETER	(b) (6)	(b) (6)	BS,PHD	PD/PI	(b) (4), (b) (6)					NA
	N	HOSSEINI, PARVIEZ RANA	(b) (6)	(b) (6)	BS,PHD	Co-Investigator						NA
(b) (6)	Y	Ross, Noam Martin		(b) (6)	PhD	Co-Investigator						NA
	N	OLIVAL, KEVIN J	(b) (6)	(b) (6)	PHD	Co-Investigator						NA
	N	KE, CHANGWEN			PHD	Co-Investigator				Center for Disease Control and Prevention of Guangdong Province	CHINA	NA
	N	ZHANG, SHUYI		(b) (6)	PHD	Co-Investigator				East China Normal University	CHINA	NA
	N	ZHANG, YUNZHI		(b) (6)	PHD	Co-Investigator				Yunnan Provincial Institute of Endemic Diseases Control & Prevention	CHINA	NA
	N	ZHU, GUANGJIAN		(b) (6)	PHD	Co-Investigator				East China Normal University	CHINA	NA
	N	GE, XINGYI			PHD	Co-Investigator				Wuhan Institute of Virology	CHINA	NA
	N	EPSTEIN, JONATHAN H	(b) (6)	(b) (6)	MPH,DVM,BA,PHD	Co-Investigator						NA
	N	CHMURA, ALEKSEI A	(b) (6)	(b) (6)	BS	Non-Student Research Assistant						NA
	N	SHI,		(b) (6)	PhD	Co-				Wuhan	CHINA	NA

		ZHENGLI				Investigator				Institute of Virology		

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

Yes

File uploaded: Noam Ross CV 2016.pdf

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

Noam Ross

(b) (6)
http://www.noamross.net
@noamross

EDUCATION

University of California — Davis, CA
Doctoral Candidate in Ecology — Expected Completion Summer 2015

- Dissertation Committee: Alan Hastings (major professor, Ecology), David Rizzo (Plant Pathology), Jim Sanchirico (Natural Resource Economics)
- Dissertation Research: "Managing Emerging Forest Disease Under Uncertainty"

Brown University — Providence, RI
Bachelor of Science in Environmental Science, Magna Cum Laude — May 2006

- Honors Thesis: "Soil Organic Matter in Northern Mongolia: Permafrost and Land-Use interactions"
- Phi Beta Kappa, Sigma Xi, Environmental Science Honors, Rosenberger Prize for Outstanding Service

SCIENTIFIC PUBLICATIONS

- Carl Boettiger*, **Noam Ross***, Alan Hastings (2013) *Early Warning Signals: The Charted And Uncharted Territories.* Theoretical Ecology http://dx.doi.org/10.1007/s12080-013-0192-6
- Fuller, Kate, David Kling, Kaelin Kroetz, **Noam Ross**, and James N. Sanchirico (2013) *Economics and Ecology of Open-Access Fisheries.* In: Shogren, J.F., (ed.) Encyclopedia of Energy, Natural Resource, and Environmental Economics, Vol. 2 Encyclopedia of Energy, Natural Resource, and Environmental Economics p.39-49. Amsterdam: Elsevier. http://dx.doi.org/10.1016/B978-0-12-375067-9.00114-5

In preparation

- (b) (4)
-

**Co-equal authorship*

POSTERS

- **Ross, Noam**. "Optimal Control of Disease in Space: An Approach Using Individual-based Models," June 1-4, 2014. 12th Annual Conference of Ecology and Evolution of Infectious Disease, Fort Collins, Colorado.
- **Ross, Noam**. "Designing Protective Treatments for Forest Disease Using a Spatial Point Process Model," November 20-21, 2014. California Forest Pest Council Annual Meeting, McClellan, CA.
- **Ross, Noam**. "Optimal Control of Forest Disease Under Changing Community and Spatial Structure," November 4-18, 2013. Sustainable Management of Natural Resources Workshop, Mathematical Biosciences Institute, Columbus, OH.

PRESENTATIONS

- **Ross, Noam**, "Fungal Disease Mortality: Modeling for Management of Sudden Oak Death." Dec 1, 2014 Invited talk at EcoHealth Alliance, New York, NY.
- **Ross, Noam**, "Modeling forest disease using a macroparasite framework ," Agust 13, 2014. 99th Annual Ecological Society of America Meeting, Sacramento, CA.
- Ashander, Jamie, Kelly Gravuer, Megan Kelso, Mary E. Mendoza and **Noam Ross** "Managing River-Floodplains Systems: A Historical and Ecological Perspective" September 14, 2002. Presentation at NSF REACH IGERT Floodplains Workshop

AWARDS + FELLOWSHIPS (*Total received $225,429*)

- Don Dahlsten Memorial Grant ($325) — California Forest Pest Council, 2012
  Designing Protective Treatments for Forest Disease Using Spatial Point Process Models
- NSF IGERT Bridge Fellowship ($57,500) — UC Davis, CA, 2012
  Managing Emerging Forest Disease Under Uncertainty
- NSF IGERT Traineeship in Rapid Environmental Change ($115,00) — UC Davis, CA, 2010
  Modifying River-Floodplain Systems: A Historical and Ecological Approach
- UC Davis Graduate Group in Ecology Fellowship ($40,604) — UC Davis, CA, 2010
- NSF Research Experience for Undergraduates Fellowship ($8,000) — Acad. of Natural Sciences, PA, 2005
- Undergraduate Research Fellowship ($4,000) — Brown University, RI, 2003

SERVICE + PROJECTS

- **Workshop Instructor,** Software Capentry and Data Carpentry Foundations — Jan 2015–Present
- **Student Rep,** UC Davis Graduate Group in Ecology Executive Committee — Sep 2013–Present
- **Reviewer:** *Theoretical Ecology* (4 reviews) — Feb 2013–Present
- **Web Developer and Technology Chair,** Ecology Graduate Student Association — June 2013–Present
  Creator + Maintainer of graduate student blog, resources, and news site (egsa.ucdavis.edu)
- **Founder + Organizer,** Davis R Users' Group — Sep 2012–Present
  Created users group that provides tutoring and seminars to graduate students in 10+ departments
- **Contributor,** R packages `knitr, knitcitations, rcrossref, rethinking` — 2012-Present
- **Organizer:** NSF REACH IGERT Workshop on Multiple Goals in Floodplain Restoration — Sep 2012
- **Organizer,** UC Davis Conference on Ecology and the Business Sector — Apr 2011
- **Organizer**, UC Davis Graduate Group in Ecology Symposium — May 2010-2011
- **External Reviewer,** World Resources Institute Corporate Ecosystem Services Review — Jan 2008
- **External Reviewer,** McKinsey-Clinton Global Initiative Forestry Project — Mar 2008
- **Business Stewardship Volunteer,** NY Coastal Marine Resources Center — Feb-Apr 2007

OTHER WORK EXPERIENCE

GreenOrder — New York, NY
Analyst, Senior Analyst: Corporate Environmental Strategy + Governance — Sep 2006–Oct 2009

- Conducted environmental performance analysis for products in energy, transportation, and water sectors
- Created green product metrics system R&D stage-gating system for construction products manufacturer
- Managed engagement with equipment rental company to identify growth opportunities in green building
- Performed market and competitive analyses for a wide array of clients in retail, real estate financial and cleantech sectors; prepared and delivered client presentations; managed projects
- Managed analysts performing environmental product certifications and market research
- Developed firm seminar series and analyst training materials; conducted trainings on topics including auditing, statistical analysis, and environmental performance benchmarking
- Audited certifications for environmental products and facility performance

Wal-Mart — Providence, RI
Contract Researcher/Consultant: Energy Efficient Products Initiative — May-Sep 2006

- Developed forecasting model for sales of energy-efficient lamps at Wal-Mart stores
- Created guidelines for design of lamp recycling program

Brown University Facilities Management Providence, RI
Administrative, Research, + Teaching Assistant: Energy and Design Jan 2003–May 2006

- Developed energy-use and financial projections for university energy usage scenarios
- Performed background research and feasibility analysis for university energy efficiency projects
- Provided tutoring, logistical support and web design for two courses in sustainable design
- Responsible for maintenance of energy efficient, low-impact building

Hovsgol Lake Global Environmental Facility and Brown University Mongolia + Providence, RI
National Science Foundation REU Fellow, Thesis Research June 2005-May 2006
Advisor: Clyde Goulden

- Independent research on climate-land use interactions on permafrost soil carbon storage
  Plant surveys, soil pit excavation, soil physical and chemical analysis, soil microbial process incubations

Marine Biological Laboratory Ecosystems Center Woods Hole, MA
Semester in Environmental Science Student Aug-Dec 2004
Advisor: Charles Hopkinson

- Examined effects of nitrogen pollution on structure of microplankton food webs
- Microcosm experiments, fluorescence microscopy, dissolved nutrient analysis, planktonic growth incubations

Brown Center for Environmental Studies Providence, RI
Undergraduate Research Fellow Jun-Aug 2003
Advisor: Steven Hamburg

- Conducted research in biogeochemistry at Hubbard Brook Experimental Forest and surrounding region; oversaw soil pit excavation by undergraduate and graduate field crew
- Plant surveys, forest floor measurements, litter collection, soil pit excavation, soil physical and chemical analysis, GIS analysis in ESRI ArcMap

PUBLICATIONS IN POPULAR PRESS

- "Extinction Debt,"(Initial author) Wikipedia. Wikimedia Foundation, Inc., February 23, 2011 http://en.wikipedia.org/wiki/Extinction_debt
- "If Everyone Moves to the City, What Gets Left Behind?" *Good.is,* January 17, 2011. http://www.good.is/post/if-everyone-moves-to-the-city-what-is-left-behind/
- "Why the Ethanol Debate Isn't Helping Anyone," *GreenBiz.com*, Jun 3, 2009. http://www.greenbiz.com/blog/2009/06/03/why-ethanol-debate-isnt-helping-anyone
- "Four Lean, Green Strategies for an Uncertain Economy," (with Andrew Shapiro) *Harvard Business Review's Leading Green*, Oct 29, 2008. http://blogs.hbr.org/2008/10/4-lean-green-strategies-for-an/
- "What a Silent Spring Means for Business Risk," *GreenBiz.com*, Mar 6, 2007. http://www.greenbiz.com/blog/2007/03/05/what-silent-spring-means-business-risk

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Dollar Amount	Country
211699	CHINA

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

No

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Report Attached: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional	Address

		District	
Primary: EcoHealth Alliance, Inc.	077090066	NY-010	460 West 34th Street 17th Floor New York NY 100012317
Wuhan Institute of Virology	529027474		Xiao Hong Shan, No. 44 Wuchang District Wuhan
East China Normal University	420945495		3663 Zhongshan Beilu Shanghai
ECOHEALTH ALLIANCE	077090066		ECOHEALTH ALLIANCE, INC. 460 W 34TH ST NEW YORK NY 100012320
EcoHealth Alliance, Inc.	077090066	NY-010	460 West 34th Street 17th Floor New York NY 100012317
Wuhan Institute of Virology	529027474		Xiao Hong Shan, No. 44 Wuchang District Wuhan
East China Normal University	420945495		3663 Zhongshan Beilu Shanghai

G.9 FOREIGN COMPONENT

Organization Name: Wuhan Institute of Virology
Country: CHINA
Description of Foreign Component:
Principal Laboratory for all Research in China as per section G8 (above) and detailed in our Specific Aims

Organization Name: East China Normal University
Country: CHINA
Description of Foreign Component:
Principal Coordinating Team for all project field work as per section G8 (above) and detailed in our Specific Aims

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 166195

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Foreign

NIH Defined Phase III Clinical Trial: No

Study Title: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

Planned Enrollment

Planned Enrollment Total: 2,460

NOTE: Planned enrollment data exists in the previous format; the PD/PI did not enter the planned enrollment information in the modified format and was not required to do so. Only the total can be provided.

Cumulative Enrollment

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	**0**
Asian	157	108	0	0	0	0	0	0	0	**265**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	0	0	0	0	0	0	0	0	0	**0**
White	0	0	0	0	0	0	0	0	0	**0**
More than One Race	0	0	0	0	0	0	0	0	0	**0**
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	**0**
Total	**157**	**108**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**265**