Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 05/26/2017

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI110964-04
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: 06/01/2017 – 05/31/2018
Project Period: 06/01/2014 – 05/31/2019

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $597,112 (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,

Laura A. Pone
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI110964-04

Award Calculation (U.S. Dollars)

Salaries and Wages	$167,708
Fringe Benefits	$54,168
Personnel Costs (Subtotal)	$221,876
Materials & Supplies	$7,000
Travel	$35,918
Other	$9,800
Subawards/Consortium/Contractual Costs	$201,422
Federal Direct Costs	$476,016
Federal F&A Costs	$121,096
Approved Budget	$597,112
Total Amount of Federal Funds Obligated (Federal Share)	$597,112
TOTAL FEDERAL AWARD AMOUNT	$597,112
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$597,112

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
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:	2017

IC	CAN	2017	2018
AI	8472350	$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) 05/25/2017
Award Processed: 05/26/2017 12:05:11 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI110964-04

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 – 5R01AI110964-04

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

SECTION IV – AI Special Terms and Conditions – 5R01AI110964-04

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
National Animal Health Laboratory, LAOS

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This Notice of Award (NoA) includes collaboration with **Wuhan University School of Public Health, CHINA.**

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This Notice of Award (NoA) includes funds for activity with **Wuhan Institute of Virology, CHINA.**

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This Notice of Award (NoA) includes funds for activity with **(East China Normal University.**

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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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This award is subject to the Clinical Terms of Award included in Monitoring of Clinical Trials and Studies - NIAID (see NIH Guide for Grants and Contracts, July 8, 2002, NOT AI-02-032). These terms and conditions are hereby incorporated by reference, and can be accessed via the following World Wide Web address: https://www.niaid.nih.gov/grants-contracts/niaid-clinical-terms-award All submissions required by the NIAID Clinical Terms of Award must be forwarded electronically or by mail to the responsible NIAID Program Official identified on this Notice of Award.

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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: Carine Normil
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: 5R01AI110964-04

INSTITUTION: ECOHEALTH ALLIANCE, INC.

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

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

A. COVER PAGE

Project Title: Understanding the Risk of Bat Coronavirus Emergence	
Grant Number: 5R01AI110964-04	**Project/Grant Period:** 06/01/2014 - 05/31/2019
Reporting Period: 06/01/2016 - 05/31/2017	**Requested Budget Period:** 06/01/2017 - 05/31/2018
Report Term Frequency: Annual	**Date Submitted:** 04/12/2017
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: 5R01AI110964-04.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: 5R01AI110964-04 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, and Zhang gave invited University and Conference lectures including Avoiding Catastrophe Meeting at Concordia Univ., Harvard Univ. Columbia Univ., National Academy of Sciences, World Humanitarian Summit in Turkey, NEIDL Symposium in Boston, Global Pandemic Policy Summit at Texas A&M Univ., One Health EcoHealth Congress in Australia, WHO briefing, Rockefeller Planetary Health meeting, 17th International Bats Conference, China National Global Virome Project Initiative Meeting, and others that included specific discussion of the current project and results.

2.Agency and other briefings: PI Daszak and Co-investigator Shi introduced this project to potential collaborators within Rockefeller Foundation, WHO, FAO, International Collaboration Bureau of Chinese Academy of Sciences, Beijing Genomic Institute, National Natural Science Foundation of China, Institute of Pathogen Biology, Chinese Academy of Medical Science & Peking Union Medical College, and Chinese CDC.

3.Public outreach: PI Daszak and Co-investigator Shi presented this work to members of NSF, NIH, U.S. CDC, the State of Forestry Administration of China, and the general public at the China National Virome Project Initiative Meeting hosted by Chinese CDC and Chinese Academy of Sciences (2017); Co-investigator Olival presented this work at the NYC Medtech Forum to the public (2016); Research Technician Dr. Guangjian Zhu presented this work at the China Conservation Expo to the conservation groups in China (2016). Co-Investigator Y-Z Zhang presented this project to the provincial infectious disease hospital Kunming No.3 People's Hospital in Yunnan province (2016).

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.

•To commence the analysis of data collected from the integrated biological behavioral surveillance questionnaires from Yunnan, Guangxi, and Guangdong provinces, linking to the viral and serological testing results of biological samples.

•Following the successful pilot of wildlife trade network research in Lipu, Guilin, Guangxi province in Year 3, we will continue the Wild Animal Farms Survey in Guangxi, and expand to Yunnan and Guangdong in Year 4, with Institutional Review Board approvals from both Yunnan Institute of Endemic Diseases Control and Prevention and Hummingbird #2016-55, to:

−Generate a network model of wildlife trade
−Model trade flows in the wildlife farmer networks to identify locations of high potential for viral recombination
−Update survey instrument for "second wave" network interviews

•We will continue the passive hospital surveillance with anonymized, surveillance data collection from acutely ill hospital in-patients who 1) satisfy syndromic eligibility criteria; 2) have complete medical records; 3) non-normative laboratory confirmed diagnostic results; and suspected acute viral infection.

Research has been successfully piloted in four hospitals in Yunnan province: 1) Dali College Affiliated Hospital; 2) Dali Prefecture Hospital; 3) Kunming No. 3 People's Hospital, and 4) Chuxiong Prefecture Hospital, 120 biological samples have been collected, with approval from the Institutional Review Boards of the School of Public Health of Wuhan University and Hummingbird IRB

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

•The genomic characterization of SL-CoVs in Year 3 was focused on Rhinolophus sinicus in Yunnan, our plan for Year 4 is to obtain complete S gene, RdRp gene or full-length genome sequences of more SL-CoVs from a broader range of bat species identified all over China and conduct a more comprehensive evolution study on SL-CoVs in bats.

•To search for the receptor of SL-CoV with deletions in the homologous region of SARS-CoV RBD (i.e. Rp3, Rs672), and SL-CoVs which has been demonstrated to be unable to utilize bat ACE2 (i.e. Rs4231) whose receptors may be some molecules other than ACE2.

•To conduct population genetics study of Rhinolophus sinicus ACE2s, which includes: the amplification of ACE2 genes from Rhinolophus sinicus samples of different origin, test of the usage efficiency of Rhinolophus sinicus ACE2s of different origins by SL-CoVs and kinetics study on the binding of SL-CoV RBD to different Rhinolophus sinicus ACE2s.

•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.

•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 3 analyses using published sequences.

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

•Using the reverse genetic method, we will construct chimeric viruses with the backbone of MERS-CoV and the S genes from diverse newly identified bat MERS-related coronaviruses, and examine the pathogenicity of bat MERS-related coronaviruses on cell and animal levels.

•The animal infection experiments are planned to be conducted in following years to study the pathogenicity of diverse SL-CoVs and MERS-related CoV that we identified in Chinese bats.

•Surveillance of infection in human populations by bat-borne CoVs in 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 nucleic acids and antibodies against the viral nucleocapsid protein or spike protein.

Year 3 Report: Understanding the Risk of Bat Coronavirus Emergence

Award Number: 5R01AI110964-04

B.2 What was accomplished under these goals?

SUMMARY

The results of the 3rd year of our R01 work are detailed below. They include:

- Initial analysis of behavioral risk qualitative research in Yunnan and wildlife market observational data in Guangdong, that suggests a reduction in wildlife hunting, trade and consumption may be underway in southern China.
- Results from a behavioral risk survey of over 1,000 people in two provinces of southern China that assesses exposure to wildlife and prior bouts of unusual illness, with concurrent taking of samples to test for evidence of exposure to SL-CoVs.
- The finding of serological evidence of spillover of bat SARS-like CoVs in 6 people in Yunnan
- Testing of over 1,000 bat samples to identify diverse alpha- and betacoronaviruses
- Full genome characterization of 26 alphacoronaviruses.
- Receptor binding domain sequences from 37 new bat SL-CoVs that shows S proteins re more diverse than previously thought.
- Host-virus co-phylogeographic analysis of a diverse group of >1,300 bat CoVs showing that these viruses have a larger host range, weaker host specificity and higher frequency of cross-genera transmission than previously thought.
- Use of our reverse genetics system to identify 3 more novel SL-CoVs with potential to directly infect people.

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

During Year 3 we began analyzing the qualitative research that was conducted in Year 2. In addition, we developed a digital application for a community-based integrated biological behavioral surveillance system and rolled this out in two provinces. The tool aims to identify specific animal exposure risk factors associated with biological evidence of exposure to SARS-like CoV (i.e. seropositive status).

Qualitative Research

Interviews conducted in Yunnan province during Year 2 were transcribed and translated into English. A total of 23 individuals (12 women; 11 men) were interviewed in rural regions where wildlife trade routes have been documented. Yunnan province was 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. The study was approved by the Institutional Review Boards of the School of Public Health at Wuhan University and Hummingbird IRB #2014-23.

Participants were recruited primarily through local contacts that have been developed as part of wildlife conservation and health research that has been ongoing in these regions in China for 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).

Educational attainment varied widely in the population; however, the majority of study participants reported limited schooling, primary education or less. This was further reflected in the occupational distribution of study participants (*Fig. 1*), while there was two respondents who reported more professional occupations, a doctor and an accountant, half (50%) were unskilled laborers or farmers, either agricultural or animal. There were one individuals who self-identified as animal farmers, farming wildlife, bamboo rat, civet, or nutria.

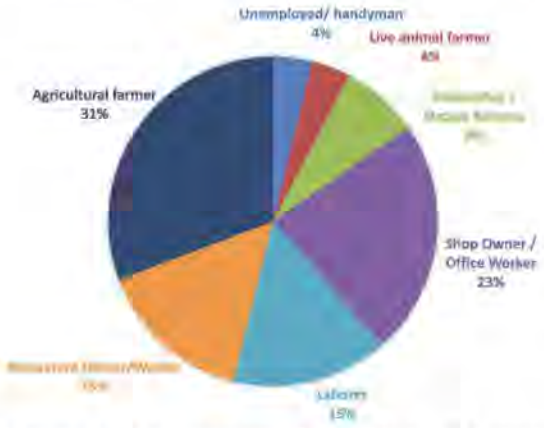

Figure 1. Occupation of Qualitative Research Participants (n=23) in Yunnan and Guangxi Provinces

Thematic analysis provided the framework with which to code and analyze data from the ethnographic interviews and focus group. Five core themes were identified to form the basis for this: (1) human-animal contact, (2) unusual illness experience and response, (3) socioeconomics and daily living, (4) biosafety and (5) human environments and movement/travel. Individual interviews and field notes were studied to ensure familiarity with the data set in its entirety and to confirm narrative consistency within individual interviews prior to coding. Using these themes and a coding keyword guide allowed for a directed and consistent coverage of the domains that were the focus of the actual interviews. Qualitative data were re-examined to develop additional theoretical categories or typologies. This analysis aims to assess perceptions, knowledge and participation in the wildlife trade, as well as barriers to participation and observed changes over time. The data were coded for factors associated with wildlife consumption, socioeconomic drivers of wildlife trade, conservation and legal efforts, the prevalence and types of wildlife observed, and wildlife exposures that could transmit disease to humans (*Table 1*).

Table 1. Topics covered in Ethnographic Interviews

Theme Discussed in Ethnographic Interview	No. of Respondents (n=23)	(%)
Work/Job Functions	22	96%
Water & Food	22	96%
Sanitation	22	96%
Hygiene	22	96%
Perceptions/Knowledge	22	96%
Home Life	21	91%
Education	20	87%
Medical Care Treatment	20	87%
Direct Contact with Animals	20	87%
Travel	19	83%
Observed Environment	19	83%
Animal Responsibilities	19	83%
Household Illness	19	83%
Indirect Contact with Animals	19	83%
Daily Routine	18	78%
Family Economics	18	78%
Illness from Animals	18	78%
Animal Health	18	78%
Animal Products/Rituals with Animal Products	16	70%
Death	14	61%

The data coding and analytic strategy was designed to avoid the need for expensive analytic software programs and to use standard word processing and spreadsheet programs readily available to in-country teams. These teams received training on qualitative data analysis, and they initiated the first phase of analyses.

Analysis focused on wildlife trade and consumption in these two provinces, specifically on how respondents perceive and contact wildlife through the changing landscape around them. The aim was to identify motivations around animal consumption and practices. A number of participants reported that wildlife are purchased as a means to impress others as a symbol of wealth. Participants routinely reported that the cost of wildlife is double or triple that of regular livestock meat. Ironically, others reported that poorer individuals in these communities who continue to eat wildlife are sometimes scorned for their poverty, because this is a habit from an older time within China. Though there is a stigma to this habit, individuals did report opportunistically capturing and consuming wildlife when convenient.

Participants also noted a decrease in wildlife over time: that in their childhood the forests would be full of the sounds of animals and birds, but this occurs no longer. This decrease was attributed to many factors, most commonly infrastructure development. Respondents discussed

the government investing resources to build new roads and renovate local infrastructure with the intention of increasing tourism, and that this has had the impact of reducing forested habitat for wildlife. Hunting and selling of wildlife was not reported by any participant as a cause of observed wildlife depletion. However, participants did attribute a reduction in wildlife hunting and consumption to an increased enforcement of conservation laws. In particular, the story of one ill-fated hunter who killed a monkey—and was caught—was reported by a number of participants from the same village.

Participants observed that the observed decrease in wildlife abundance and increased conservation law enforcement has made it more difficult to make a living from the wildlife trade. Participants reported choosing alternative forms of money making, indicating that only those people who belong to low socioeconomic classes continue to hunt secretly. The cost-benefit analysis that pits the threat of punitive consequences against the profits to be made through wildlife hunting are only feasible for those 'who have nothing to lose.'

Table 2: Species Observed in Wet Markets 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
Rattus norvegicus	Common Rat

Observations by research staff in live animal markets in Guangzhou found wildlife to be plentiful (*Table 2*), 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.

Integrated Biological Behavioral Surveillance in Yunnan and Guangdong Provinces

To better assess the mechanisms of zoonotic viral spillover, and build on data acquired via ethnographic interview (above) we have designed a structured behavioral questionnaire to measure both exposure and outcome data. This behavioral risk survey assesses exposure to wildlife and bouts of unusual illness over a respondent's 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 SL-CoVs have been previously detected in the bat population in Jinning County, Yunnan. After the questionnaire was pilot tested and optimized to fit the research aim, the survey was developed as a digital application (https://www.dropbox.com/s/sv62neywuvl027r/Questionnaire%20Complete.docx?dl=0%). This allows standardization across all field teams and quality control. Four field team leads were trained on behavioral survey data collection, data collection technologies (the digital application) and analysis. The questionnaire was then administered in a follow-up survey in Yunnan province and then in Guangdong province. Surveillance in Guangxi is currently underway.

Of 1089 participants who completed the behavioral questionnaire, 660 (61%) were women and 424 (39%) were men (5 missing for this variable), with a mean age of 50 (range: 10-99). Most reported being farmers (79%) (*Fig. 2*), a majority were long term residents (97%) and 41% had a family income under 3000 RMB annually ($430). Almost three quarters (72%) of the respondents have had only primary level education or less.

Figure 2. Occupation of Integrated Biological Behavioral Surveillance Participants in Yunnan and Guangdong Provinces

Standardized syndromic case definitions informed questions concerning unusual illness experience (e.g. severe acute respiratory infections [SARI], influenza-like illness [ILI], febrile symptoms [Encephalitis]). Lifetime, 12 month, and unusual illnesses experienced in the family for the past 12 months were assessed for all participants. In the past year, SARI was reported by 55 (5.1%) respondents and 14 of those respondents also responded SARI symptoms in family members (*Table 3*).

Table 3. Unusual Illness Experience In Respondents Lifetime, Past 12 months, Family members

Symptoms	Ever	Past 12 months	Family Past 12 months
Severe Acute Respiratory Infections (SARI)	118 (10.8%)	55 (5.1%)	40 (3.7%)
Influenza Like Illness (ILI)	305 (28.0%)	128 (11.8%)	142 (13.0%)
Encephalitis	98 (9.0%)	52 (4.8%)	30 (2.8%)
Hemorrhagic Fever	2 (0.2%)	2 (0.2%)	0 (0.0%)
Fever with Diarrhea /Vomiting	58 (5.3%)	25 (2.3%)	21 (1.9%)
Fever with Rash	10 (0.9%)	7 (0.6%)	7 (0.6%)

Type of exposure and species exposed to are shown below (*Table 4*). Poultry was the most commonly contacted animal in almost all categories. Three quarters of respondents reported rodents or shrews entering their home in the past 12 months.

Table 4: Animal Species Contact by Type of Contact

	Pets	Handled	Raised	In house	Cooked/ handled	Eaten raw/ under-cooked	Found dead collected	Scratched/ bitten	Slaughtered	Hunted/ trapped
Rodents/Shrews	0	33	5	834	38	2	1	1	28	26
Bats	0	5	0	180	8	0	0	1	5	5
Non-human primates	0	1	3	7	4	0	0	0	1	1
Birds	3	19	8	497	39	3	0	0	12	12
Carnivores	1	16	7	100	36	0	0	0	19	10
Ungulates	0	5	12	23	50	0	0	0	8	1
Poultry	5	514	843	134	719	5	8	6	425	7
Goats/Sheep	0	16	38	4	80	1	0	0	17	0
Swine	3	210	494	43	533	47	1	1	147	2
Cattle/Buffalo	0	12	77	10	102	5	1	0	11	1
Dogs	342	40	303	252	62	0	0	22	16	2
Cats	163	10	137	275	18	0	0	11	1	0

Animal exposures among those who reported unusual illness experiences in the past 12 months were evaluated, focusing on three high interest syndromes: SARI, ILI, and encephalitis. Of the 55 respondents who reported SARI symptoms, 49 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. 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 a wet market.

Table 5. Self-Reporting Symptoms of Syndromes and Sociodemographic and Animal Contact.

	SARI Positive *n=55*		ILI Positive *n=128*		Encephalitis Positive *n=52*	
Sociodemographics	n	%	n	%	n	%
Mother Primary education or less	54	98.2%	121	94.5%	50	96.2%
Primary education or less	45	81.8%	94	73.4%	38	73.1%
Female	32	58.2%	74	57.8%	29	55.8%
Income <3000RMB	30	54.5%	45	35.2%	23	44.2%
Travel (past 12m)	30	54.5%	69	53.9%	34	65.4%
Children < 5 yrs in Household	15	27.3%	38	29.7%	17	32.7%
Household member with same syndrome	14	25.5%	46	35.9%	10	19.2%
Respondent age <35	6	10.9%	24	18.8%	14	26.9%
Animal Exposures	n	%	n	%	n	%
Come in home	50	90.9%	117	91.4%	50	96.2%
Raise animals	49	89.1%	113	88.3%	48	92.3%
Prepare/cook recently killed	37	67.3%	95	74.2%	35	67.3%
Handle live	36	65.5%	72	56.3%	38	73.1%
Slaughtered	31	56.4%	57	44.5%	34	65.4%
Animals as Pets	23	41.8%	55	43.0%	28	53.8%
Buy Animals at Wet Market	16	29.1%	49	38.3%	4	7.7%
Shared water source	9	16.4%	13	10.2%	12	23.1%
Feces in/near food	8	14.5%	9	7.0%	8	15.4%
Consume raw/undercooked	7	12.7%	10	7.8%	9	17.3%
Scratch/bite	4	7.3%	2	1.6%	4	7.7%
Consume food damaged by animals	3	5.5%	5	3.9%	2	3.8%
Hunt or Trap	2	3.6%	4	3.1%	7	13.5%
Collect dead wildlife	1	1.8%	1	0.8%	1	1.9%
Consume sick animals	0	0.0%	1	0.8%	0	0.0%

We examined the sociodemographic attributes and the types of contacts that were reported in those who reported SARI, ILI, or encephalitis-like symptoms in the past year (see *Table 5*). Over 65% of respondents these syndromes and also reported raising animals, animals coming in the home, or preparing meat or organs from a recently killed animal. A quarter of those who reported symptoms consistent with that of encephalitis were under the age of 35.

Respondents were asked about the source of their unusual illnesses. None reported any kind of animal exposure as a potential source of infection and 11% did not have any idea what may have caused their previous infection, despite the fact that a majority of respondents who reported SARI, ILI, or encephalitis symptoms also reported animal exposures (Table 5). Just over 30% of respondents reported purchasing live animals from a wet market in the past year. Over half (582; 53%) of respondents were worried about disease or disease outbreaks in animals at wet markets and 56% of people believe that animals spread disease. However, those who had purchased animals from markets in the last 12 months reported a great deal of behavior change being undertaken. In particular, respondents reported buying live animals less often 33%, only buying farmed wildlife 32% or buying meat at the supermarket 30% (*Table 6*). For those who participated in animal slaughter or were scratched or bitten in the past year, only 48 respondents (9.9%) reported visiting a doctor.

Table 6: Behavior Change at Wet Market in the last 12 months

Behavior	n	%
Wash hands	119	33.4%
Buy live animals less often	119	33.4%
Buy only farmed wildlife	113	31.7%
Sometimes shop for meat at supermarket	107	30.1%
Wear gloves	7	1.9%
Wear a mask	5	1.4%

Serological Evidence of Bat SARS-Like CoV Infection in Humans

Along with the behavioral survey questionnaire, respondents were also asked to provide a biological sample to assess SARS CoV spillover at the high-risk location where the questionnaire has been implemented.

A sensitive and specific ELISA method was developed using the recombinant bat SL-CoV Rp3 NP protein to detect SL-CoV IgG antibodies. Six (2.8%) serum samples from 218 village residents who lived closely to the bat colonies in Yunnan where we isolated SL-CoV WIV1 and WIV16 were positive for SARS-like CoV antibodies (*Fig. 3*). The 6 ELISA positive samples were further confirmed as anti-SL-CoV NP IgG positive by western blot using recombinant Rp3-NP as antigen (*Fig. 4*).

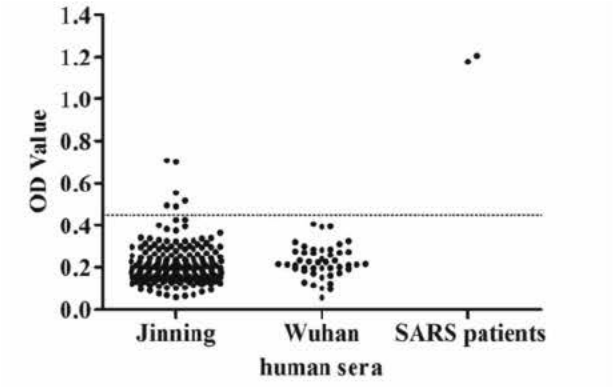

Figure 3. Serum samples from Jinning, Wuhan, and SARS patients were screened for reactivity of Rp3-NP. Bar in the diagram indicates optical density (OD) cutoff value (0.45) based on healthy blood donors in Wuhan.

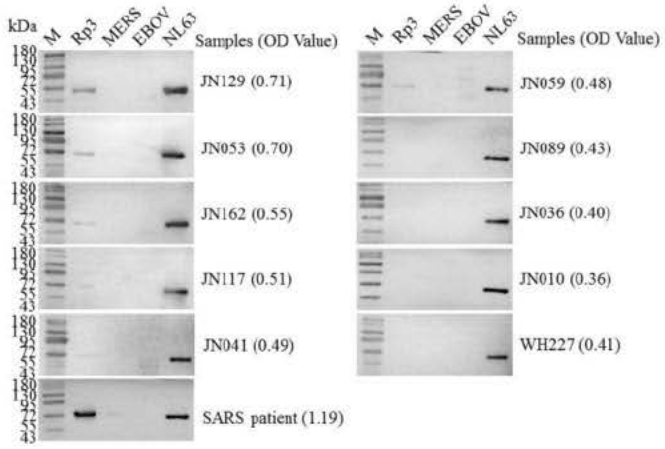

Figure 4. Western blot analysis of reactivity of human sera to Rp3-NP.

Linking Serological Findings with Respondent Questionnaire Data

Of the 6 respondents in Jinning, Yunnan with serological evidence of SL-CoV infection, 4 had handled animals, 3 had raised or cooked meat from recently killed animals, 2 found animal feces near food stuffs, and 1 slaughtered or hunted an animal. Three of the individuals had contact with poultry in the past twelve months and 2 had contact with either birds, swine or buffalo. One individual reported having contact with a bat. Responses to the questionnaire show that in the last twelve months all of the respondents who have positive testing results, had animals in their dwelling and had contact with rodents or shrews. All 6 of the respondents had reported purchasing an animal from a wet market in the past twelve months.

In addition, 215 oral swabs and 212 rectal swabs collected from human participants in Jinning and Yunnan province were tested for CoV RNA, and no positive results were found. 534 oral swabs, 526 rectal swabs from Xishuangbanna, Yunnan province; and 419 oral swabs, 412 rectal swabs from Ruyuan and Zengcheng, Guangdong province are being tested for CoV.

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

Bat CoV PCR Detection and Sequencing from Live-Sampled Bat Populations

We collected 893 rectal swab samples, 167 fecal samples and 33 blood samples from at least 17 bat genera in Yunnan, Guangdong, Guangxi, Hubei and Tibet provinces (*Table 7*) in Year 3. During this year, overall 1060 samples were tested for CoV RNA and 130 (12.3%) were positive (*Table 8*).

Table 7. Bat samples collected for CoV surveillance in Year 3

Date of Sampling	Sampling Locations	Rectal swab	Fecal pellet	Blood specimen
May 11th 2016	Mengla, Yunnan	32	--	9
May 16th 2016	Jingna, Yunnan	16	114	13
May 22nd 2016	Lufeng, Yunnan		53	--
June-July, 2016	Shixing county, Shaoguan, Guangdong	113	--	--
July 2016	Qingzhangshan, Shaoguan, Guangdong	101	--	--
July 10th 2016	Ruyuan, Guangdong	16	--	--
July 11th 2016	Chengjia, Nanling, Guangdong	26	--	--
July 2016	Huadu, Guangzhou, Guangdong	29	--	--
August 6th 2016	Lengshuitang village, Guilin, Guangxi	135	--	--
August 6th 2016	Nanxishan Park, Guilin, Guangxi	31	--	--
August 9th 2016	Lanwu village, Ruyuan, Guangdong	53	--	--
August 10th 2016	Liangkou twon, Conghhua, Guangdong	32	--	--
August 13th 2016	Jinning, Yunnan	34	--	--
August 14th 2016	Lufeng, Yunnan	25	--	--
August 16th 2016	Jingna, Yunnan	33	--	--
August, 2016	Menghai, Yunnan	125	--	--
August 21st 2016	Yaoqu village, Mengla, Yunnan	30	--	--
September, 2016	Wuhan, Hubei	36	--	--
September, 2016	Motuo, Tibet	26	--	11
Total		**893**	**167**	**33**

Genetically diverse alphacoronaviruses related to bat coronavirus 1A/1B, HKU7, HKU6 and HKU2 were identified in *Miniopterus*, *Myotis* and *Rhinolophus* bats, respectively. A novel alphacoronavirus related to human coronavirus NL63 was detected in *Tylonycteris robustula* in Yunnan. SARS-like coronaviruses were detected in 14 Chinese horseshoe bats (*Rhinolophus sinicus*) in Yunnan and Guangdong. Betacoronaviruses related to HKU5 were found in *Pipistrellus abramus* from Hubei, while two lineages of HKU4-related viruses were identified in two species of *Tylonycteris* bats in Yunnan (*Fig. 5*).

Table 8. CoV testing results for bat samples collected in Year 3

Species	Yunnan	Guangdong	Guangxi	Hubei	Tibet	Total
Rousettus spp.	1/34				6	1/40
Aselliscus stoliczkanus	31					31
Rhinoluphus spp.	16/41	11/136	6/60		5	33/242
Hipposideros spp.	17	1/126	6		8	1/157
Myotis spp.	7	6/34	7/69	1		13/111
Chaerephon spp.	8					8
Megaderma spp.	2				1	3
Ia io	1					1
Tylonycteris spp.	32/124	8				32/132
Pipistrellus spp.	1	45		5/35	2	5/83
Eonycteris spelaea	1/29					1/29
Nyctalus velutinus		2				2
Coelops spp.		2				2
Miniopterus spp.		9/17				9/17
Taphozous melanogopon			31			31
Cynopterus sphinx					3	3
Murina spp.					1	1
Fecal pellets	35/167					35/167
Sub-total	**85/462**	**27/370**	**13/166**	**5/36**	**0/26**	**130/1060**

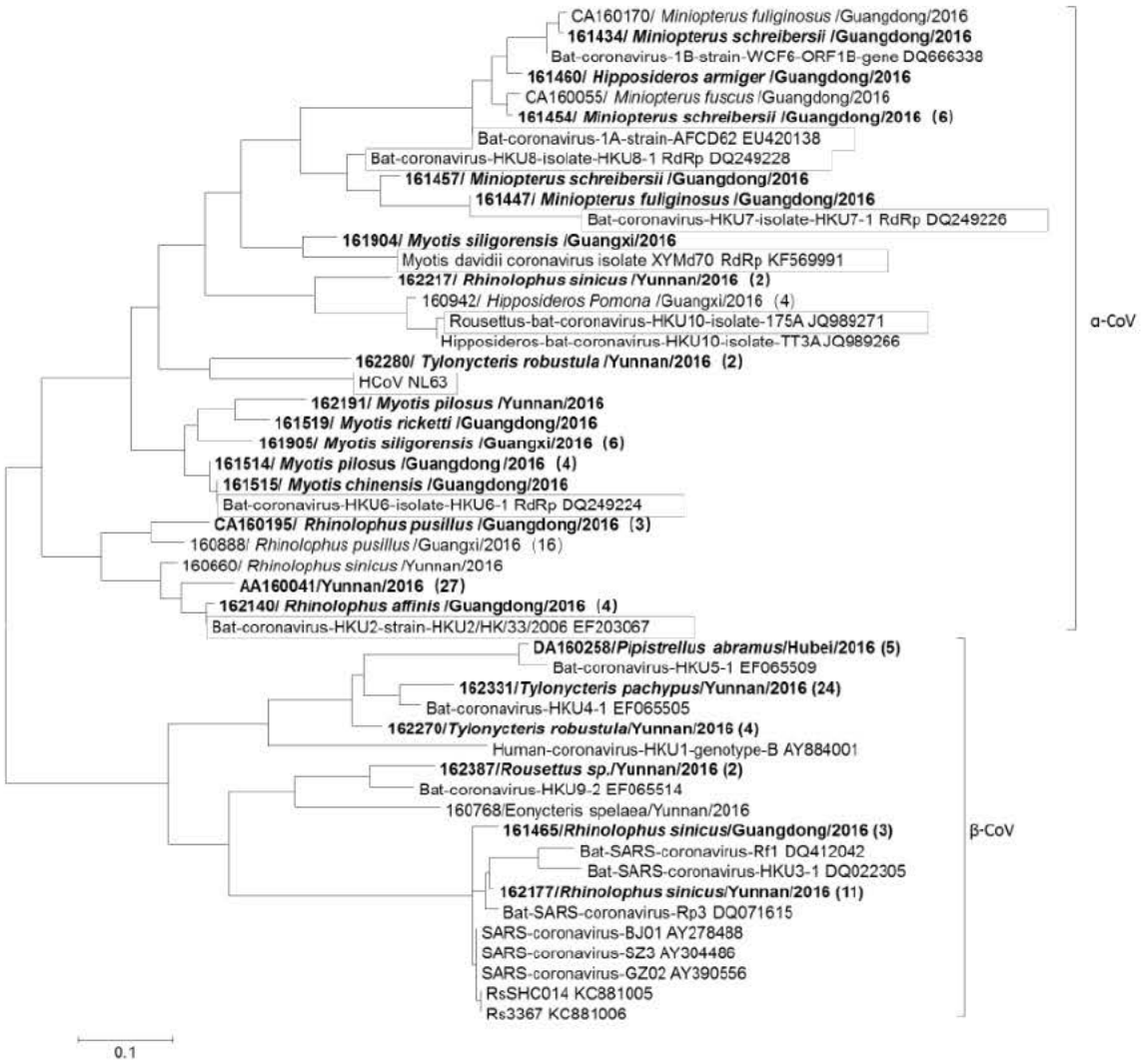

Figure 5. Phylogenetic analysis of partial RdRp gene of CoV (440-nt partial sequence).

Genomic Characterization of Novel Bat Alpha- Coronaviruses

We generated full-length genome sequences of 26 novel alphacoronaviruses from multiple *Hipposidoeros*, *Rhinolophus* and *Hypsugo* bat species. These alphacoronaviruses grouped into 4 different lineages, including HKU10-like CoVs and 3 novel species according to criteria generated by the International committee of Taxonomy of Viruses (ICTV) (*Fig. 6*). Strains belonging to the novel lineage from *Rhinolophus* share highly similar genome structures with each other but are distinct from all previously sequenced alphacoronaviruses. Putative 3b and 3c genes were identified at the upstream of the E gene, and a 7b gene at the downstream of the N gene was a homologue to *Rhinolophus* bat SARS-like CoV 7a gene. These results expand the understanding of genetic diversity of bat alphacoronaviruses.

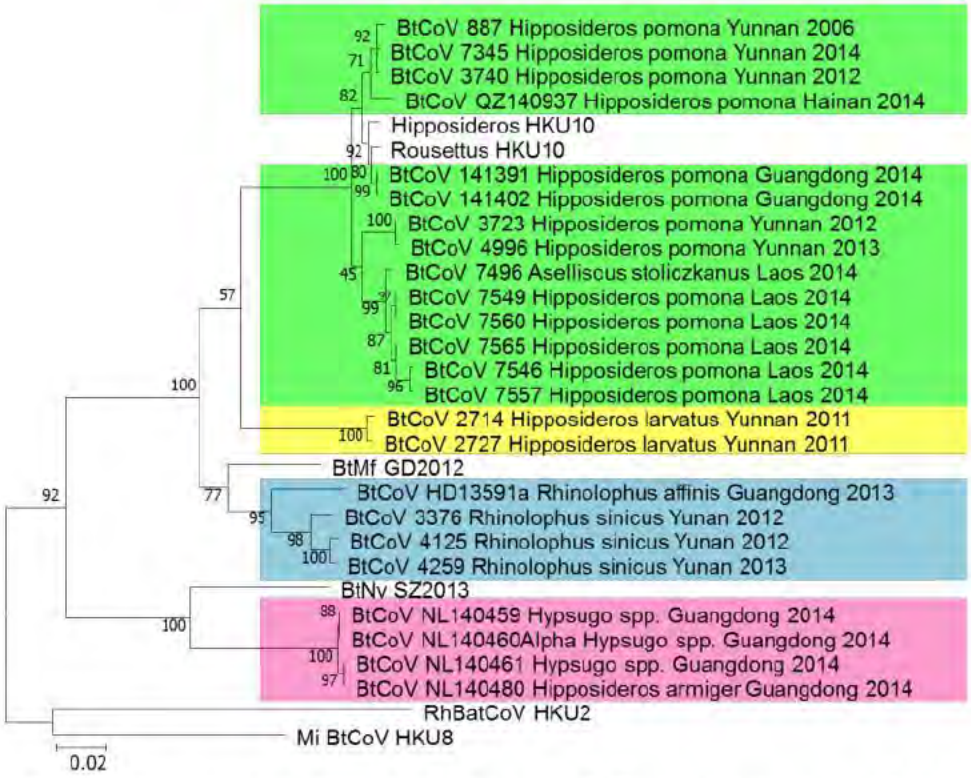

Figure 6. Phylogenetic analysis based on full-length RdRp gene sequence of alpha-CoVs

Genetic Diversity of Receptor-Binding Domain (RBD) of SARS-Like Coronavirus in Chinese Bats

RBD sequences from 37 newly identified SL-CoV from various horseshoe bat species and *Hipposideros* bat species in Yunnan, Guangdong, Guangxi, Hubei and Hunan provinces were amplified and sequenced in Year 3. Phylogenetic analysis revealed that SL-CoV circulating in bat populations in China are highly diverse in the RBD region (*Fi.g 7*). Some strains possessed an RBD sequence distinct from all currently known bat SL-CoVs and formed a new cluster in the phylogenetic tree. However, except for a few strains from Yunnan, most of these SL-CoVs contained nucleotide deletions and were relatively distant to SARS-CoV in the RBD region. These findings suggest that the S gene of SL-CoVs in Chinese bats is even more genetically diverse than expected.

The genomic characterization of SL-CoVs in Year 3 was focused on *Rhinolophus sinicus* in Yunnan, our plan for Year 4 is to obtain complete S gene, RdRp gene or full-length genome sequences of more SL-CoVs from a broader range of bat species identified all over China and conduct a more comprehensive study of the evolution of SL-CoVs in bats.

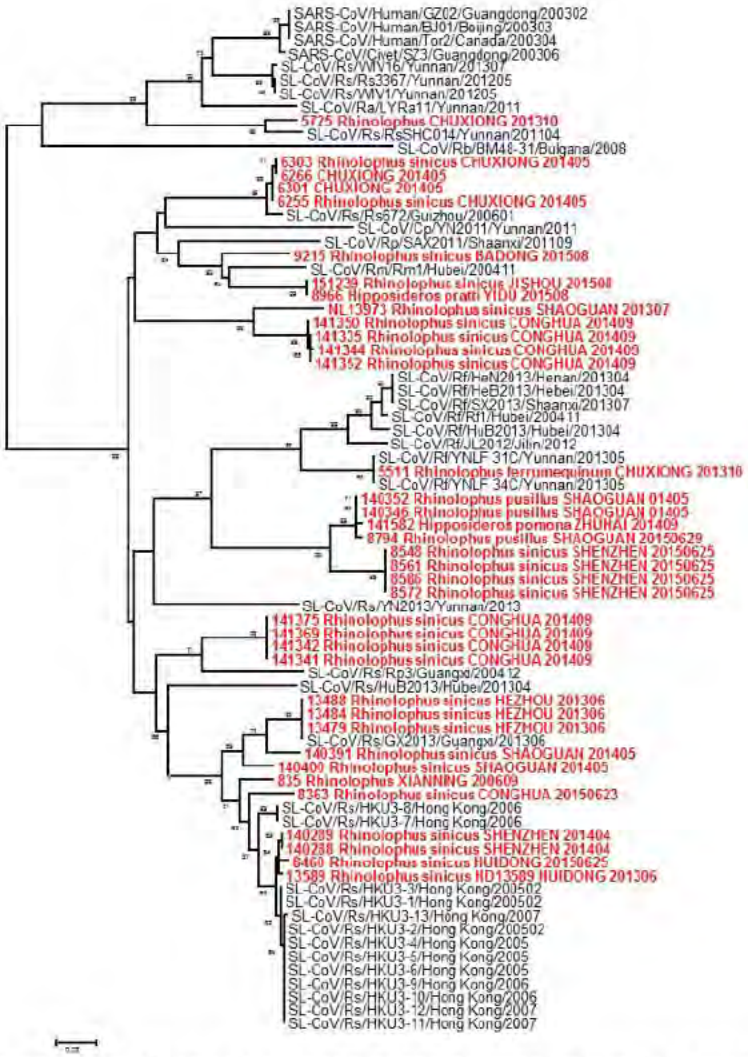

Figure 7. Phylogenetic analysis of the RBD region of the S gene of bat SL-CoVs detected in China (newly identified sequences were marked in red).

Bat Coronavirus Host-virus Phylogeography in China

To analyze the extent to which different bat species and genera are host to similar bat-CoVs, we reconstructed viral phylogenetic relationships and mapped host-species associations onto these phylogenies. Our dataset includes all CoV RdRp sequences isolated from bat specimens collected by our team from 2008-2015 (Alpha-CoVs: n = 491 – Beta-CoVs: n = 326), including those collected under prior NIAID funding (1 R01 AI079231), and funding from Chinese Federal Agencies. All Chinese bat CoV RdRp sequences available in GenBank were also added to our dataset (Alpha-CoVs: n = 226 – Beta-CoVs: n = 206). Phylogenetic trees were reconstructed for Alpha- and Beta-CoVs separately using Bayesian inference and Maximum Likelihood (ML) approaches. RAxML was used to perform ML analysis and Bayesian analyses were performed with MrBayes 3.2.6.

Beta-CoV sequences clustered into four main genetic lineages: B (SARS-CoV and SARS-like CoVs), C (MERS-CoV), D and a potential new lineage related to lineage B (*Fig. 8*). An important phylogenetic structure is observed within lineages C and D. Alpha-CoV sequences clustered into numerous closely related and less-differentiated lineages (*Fig. 9*).

We observed significant CoV lineage sharing among bat genera in our phylogenetic trees. Importantly SARS-like CoVs (SL-CoVs in lineage B) have been detected in Hipposideridae bats in addition to Rhinolophidae bats which were thought to be the putative natural host taxa of SL-CoV (*Fig. 8*). We found additional bat genera that also hosted CoVs in this clade (*Fig. 8*), expanding potential host targets for novel SL-CoV discovery. CoVs closely related to Bat coronavirus HKU9 (lineage D), which were thought to be specific to pteropodid bats, have also been detected in hipposiderid and vespertilionid bats (*Fig. 8*). Important lineage sharing across several bat families has also been observed among most Alpha-CoV lineages (*Fig. 9*). We used host DNA barcoding to confirm these findings - host mitochondrial sequences were generated to confirm the host species identity for most samples.

These results indicate a larger host range, weaker host specificity and higher frequency of cross-genera transmission for most bat CoV lineages than previously thought. These findings will have important implication in our understanding of bat CoV emergence and spillover risk in China. In Year 4 we will expand these analyses to include more explicit co-evolutionary analyses to identify the frequency and timing of host switching events for each major clade.

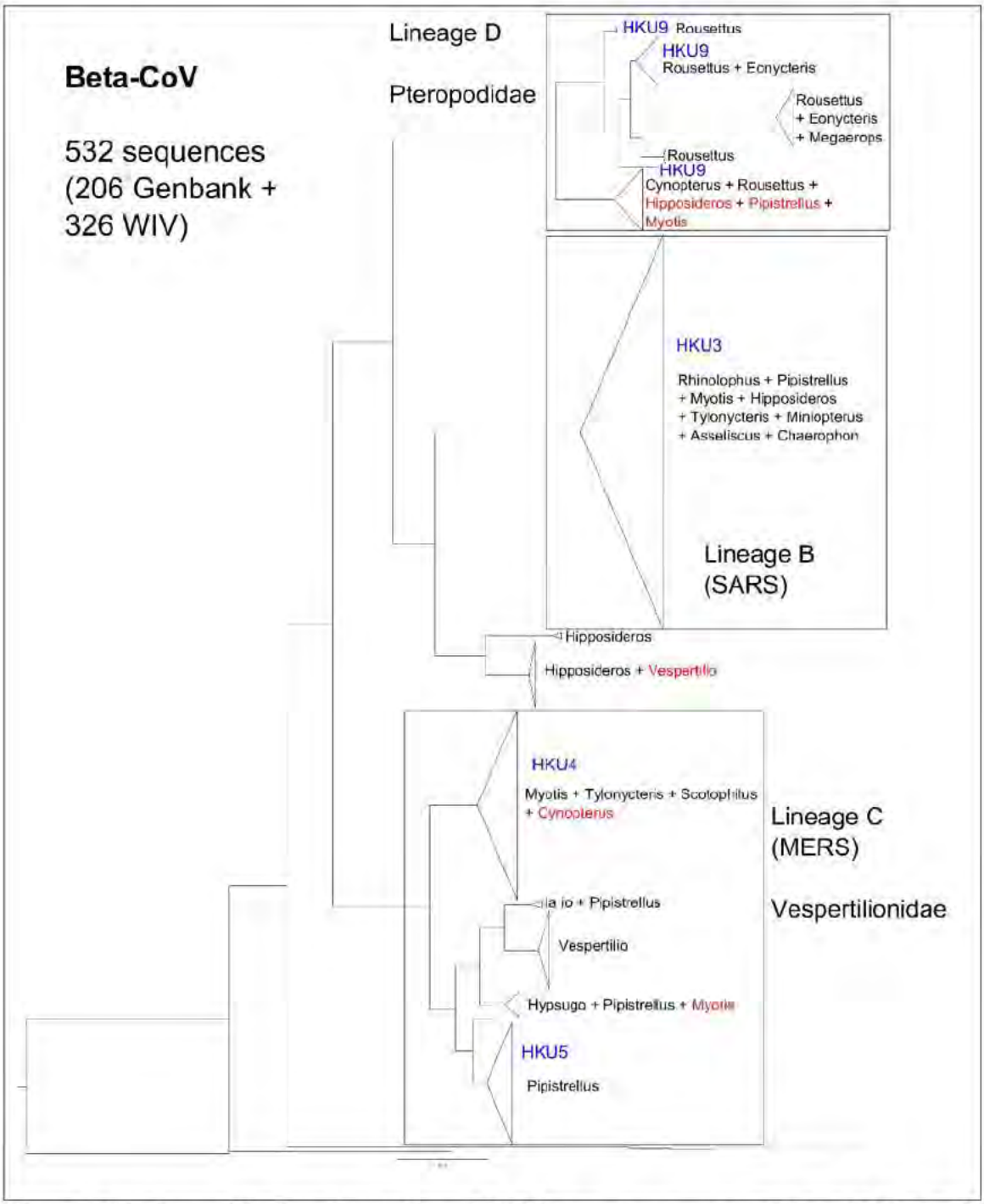

Figure 8. Maximum Likelihood tree of partial RdRp gene sequences of Beta-CoVs. Bat host genera are indicated along each lineage. Bat genera listed in red correspond to minor and potential new bat hosts and may represent cross-genera/family transmission events.

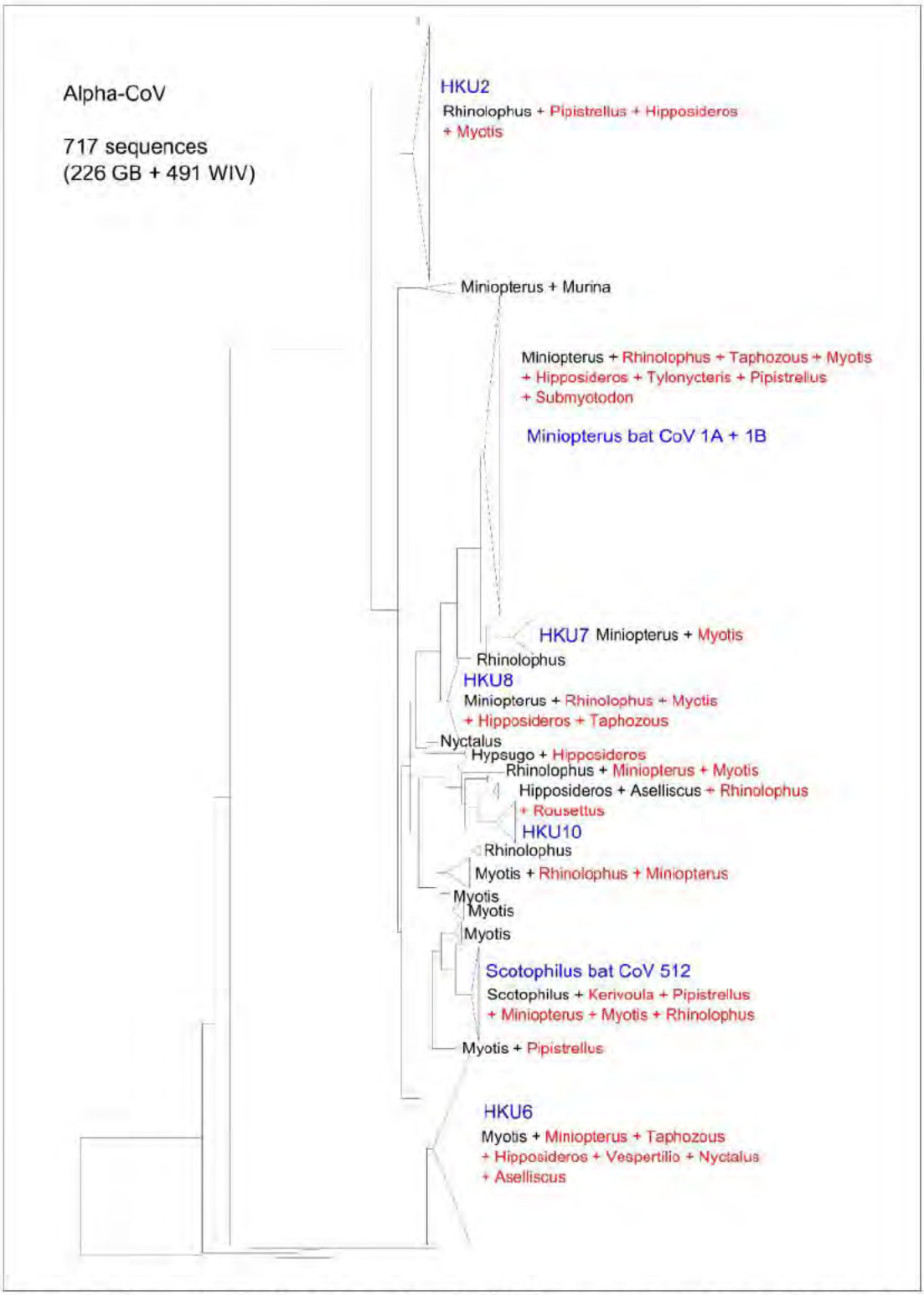

Figure 9. Maximum Likelihood tree of partial RdRp gene sequences of Alpha-CoVs. Bat host genera are indicated along each lineage. Bat genera listed in red correspond to minor and potential new bat hosts and may represent cross-genera/family transmission events.

Global analysis of bat viral sharing to identify key host species

We curated and analyzed a global dataset of bat host–virus associations to better understand the frequency, and connectivity of viral sharing among bats. We also used this to examine the importance of cave-roosting bats species in harboring and sharing viruses with non cave-roosting species, and to identify specific hosts that are central in the network (*Fig. 10*). Cave roosting bat species are host to most CoVs found in bats (orange). We identified global patterns of viral coinfection based on the number of connections between each virus in the network (Fig. 10). We will expand this approach to our China-CoV specific field data in Year 4.

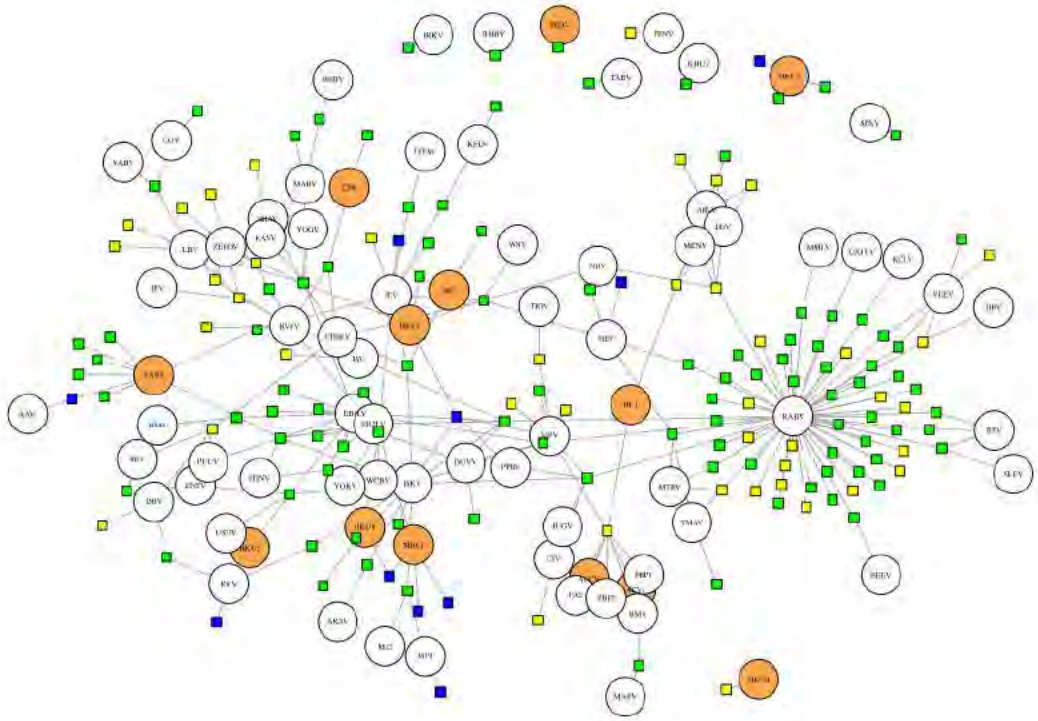

Figure 10. An analysis of global bat virus sharing using data from the published literature combined with field data. Network analysis includes 152 bat host species and 80 ICTV recognized viral species, with 273 host-viral associations. Unique viruses are represented in circles with known CoVs shown in orange, and each square represents a unique bat species. Green squares = facultative cave-roosting bat species; Blue squares = obligate cave-roosting species; Yellow squares = non cave-roosting species. Viruses are linked in the network based on host species that have been observed harboring the same virus – as detected using PCR or viral isolation.

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

In Year 3 we established an effective and economic reverse genetics system for bat SL-CoV which can be applied to efficiently rescue SL-CoVs that are difficult to culture. This can be used to explore the functions of newly identified SL-CoV genes, as well as to assess pathogenesis of novel bat SL-CoVs. Using this system, we demonstrated that the unique ORFx in WIV1 and WIV16 is a functional gene involving modulation of the host immune response but not essential for *in vitro* viral replication (Zeng et al, 2016, J Virol).

Identification of Three Novel SL-CoVs with Potential for Direct Transmission to Humans
In Y2, we conducted full-length genome sequencing of 11 novel SL-CoVs detected in a single bat habitat in Yunnan province, which included strains highly similar to human/civet SARS-CoV in the most variable genes (N-terminal domain and RBD in the S gene, ORF8 and ORF3) (under revision). Based on recombination analysis, we hypothesized that the direct progenitor of the pandemic SARS-CoV may originated from this location after sequential recombination events at multiple genomic positions.

Among the 11 newly identified SL-CoVs, three different strains namely Rs4874, Rs7327 and Rs4231 contained no deletions in the RBD region but their RBD sequences varied from each other. Rs4874 has an S gene almost identical to that of WIV16. Rs7327's S protein varies from that of WIV1 and WIV16 at three aa residues in the receptor-binding motif, including one contact residue (aa 484) with human ACE2. Rs4231 shares similar NTD sequence with WIV1 and WIV16, but has a distinct RBD sequence. In Year 3, we successfully isolated Rs4874 from the single fecal sample. Using the reverse genetic system we previously developed, we constructed two chimeric viruses with the WIV1 backbone replaced with the S gene of Rs7327 and Rs4231, respectively. Vero E6 cells were respectively infected with Rs4874, WIV1-Rs4231S and WIV1-Rs7327S, and efficient virus replication was detected by immunofluorescence assay in all infections. To assess the usage of human ACE2 by the three novel SL-CoVs, we conducted virus infectivity studies using HeLa cells with or without the expression of human ACE2. All viruses replicated efficiently in the human ACE2-expressing cells. The results were further confirmed by quantification of viral RNA using real-time RT-PCR (*Fig.11*).

<u>These finding suggests that diverse variants of SL-CoV S protein without deletions in their RBD are able to use human ACE2 as receptor for cell entry. Diverse SL-CoVs capable of direct transmission to humans are circulating in bats in southwestern China, which represents a potential risk of emergence given the opportunity to spillover to other animals and/or human populations</u>.

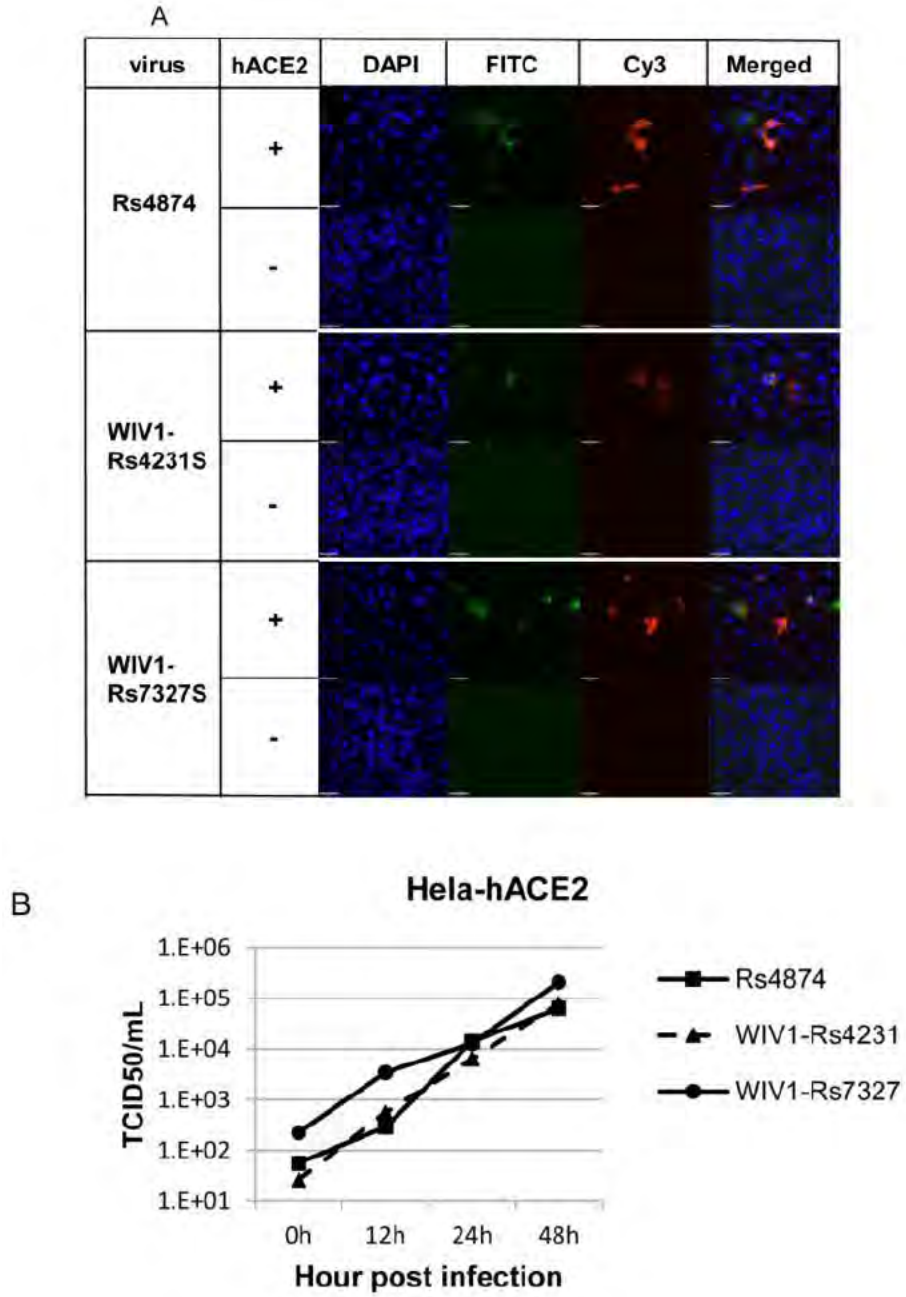

Figure 11. Analysis of receptor usage by immunofluorescence assay (A) and real-time PCR (B).

Additional Year 3 items for Specific Aim 3:

- The full-length infectious cDNA clone of MERS-CoV has been successfully constructed. The full-length S gene of 12 different novel bat MERS-related coronaviruses have been amplified and cloned into the T-vectors. In Y4, we aim to use the reverse genetic method, and construct chimeric viruses with the backbone of MERS-CoV and the S genes from

diverse newly identified bat MERS-related coronaviruses, to examine the pathogenicity of bat MERS-related coronaviruses on cell and animal levels.

- Establishment of animal infection models for bat SL-CoV and MERS-related CoV: Mice with human ACE2 have been imported to China and have been bred for one generation in Wuhan Institute of Virology. Transgenic mice that express human DPP4 have also been constructed and are being bred. The animal infection experiments are planned to be conducted in following years to study the pathogenicity of diverse SL-CoVs and MERS-related CoV that we identified in Chinese bats.

Specific Goal Not Meet

- Observations and animal sampling at wildlife markets were not done in Year 3 because the stricter law enforcement and subsequent cautiousness of traders make it difficult to access to wild animal in markets. Instead, we piloted the wild animal farm survey and will be focusing on it in Year 4, with evidence from pre-investigations that shows most wild animal farms serve as transit points during the wildlife trade.
- The passive hospital surveillance has been piloted in Year 3 and will continue in Year 4 to collect and test samples for SL-CoV and other viral families
- Cophylogenetic analyses of bat host and CoV phylogenies to assess patterns of evolutionary congruence and frequency of cross-species transmission to be continued in Year 4
- Animal infection experiments of SL-CoVs and MERS-related CoV were not done in Year 3, as this is planned as part of work in Year 4.

Significant Oral Presentations

1. Daszak P. Plenary talk, One Health-EcoHealth Congress, Melbourne, Dec. 2016
2. Daszak P. 2nd annual Global Pandemic Policy Summit, Scowcroft Ctr, Texas A&M Univ.
3. Daszak P. Global Health Security Agenda side event, UN World Humanitarian Summit: FAO/WHO/USAID/Global He@lth 2030 Innovation Task Force; Istanbul, Turkey.
4. Daszak P. Symposium at École du Val-de-Grâce, Paris
5. Daszak P. Plenary, Institute of Zoology symposium on Bushmeat and disease risks, London.
6. Daszak P. Duke University Provost's Forum on Conservation and Health
7. Olival KJ. The 17th International Bat Research Conference "Assessing the Risk of Disease Emergence from Bat Hunting: Overview and Implications for Risk Mitigation". Durban, South Africa, 2016
8. Daszak P. American Public Health Association Annual Meeting 2016 "Preliminary Results from An Innovative One Health Behavioral Surveillance System". Denver, 2016

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

We presented this work to the chief physicians, nurses, and directors from county-level clinics in Guangdong and Yunnan provinces during the implementation of Integrated Biological Behavioral Surveillance in Chuxiong and Guangzhou. All the research staff were trained and re-trained for the biosafety and PPE use for human biological sampling.

11 graduate students from School of Public Health of Wuhan University and Wuhan Institute of Virology of CAS were trained for laboratory and field biosafety and PPE use, behavioral data collection methodologies and technologies, and data analysis.

Research Technician Dr. Guangjian Zhu was invited by the Institute of Pathogen Biology, Chinese Academy of Medical Science & Peking Union Medical College to provide training to 10 field team members regarding biosafety and PPE use, bats and rodents sampling.

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
Non-Compliant	(b) (4)
Complete	Zeng LP, Gao YT, Ge XY, Zhang Q, Peng C, Yang XL, Tan B, Chen J, Chmura AA, Daszak P, Shi ZL. Bat Severe Acute Respiratory Syndrome-Like Coronavirus WIV1 Encodes an Extra Accessory Protein, ORFX, Involved in Modulation of the Host Immune Response. Journal of virology. 2016 July 15;90(14):6573-82. PubMed PMID: 27170748; PubMed Central PMCID: PMC4936131.
Complete	Olival KJ, Willoughby AR. Prioritizing the 'Dormant' Flaviviruses. EcoHealth. 2017 March;14(1):1-2. PubMed PMID: 28194584; PubMed Central PMCID: PMC5386397.

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

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

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

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?

No

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

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
213239	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
Wuhan University School of Public Health	549376772	00-000	115 Donghu Road Wuhan nullnull

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: Wuhan School of Public Health
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	708	459	0	0	0	0	0	0	0	**1167**
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	**708**	**459**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1167**