



Grant Number: 5R01AI110964-02
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: [REDACTED] (b) (6)

Period Of Performance:

Budget Period: 06/01/2015 – 05/31/2016

Project Period: 06/01/2014 – 05/31/2019

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of \$630,445 (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

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 Central Contractor Registration. 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/>.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI110964-02

This Notice of Award (NoA) includes funds for consortium activity with **Wuhan Institute of Virology - CHINA** awarded in the Total Costs amount of **\$139,015 (\$128,718 Direct Costs + \$10,297 F&A Costs)**.

Future year commitments are as follows:

Year 3 Total Costs: \$159,122
Year 4 Total Costs: \$159,122
Year 5 Total Costs: \$159,122

This Notice of Award (NoA) includes funds for consortium activity with **East China Normal University - CHINA** awarded in the Total Costs amount of **\$72,684 (\$67,300 Direct Costs + \$5,384 F&A Costs)**.

Future year commitments are as follows:

Year 3 Total Costs: \$54,117
Year 4 Total Costs: \$42,300
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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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/bmb15/bmb15toc.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:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- o 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:** Laura A. Pone

**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:** 5R01AI110964-02

**INSTITUTION:** ECOHEALTH ALLIANCE, INC.

| Facilities and Administrative Costs | Year 2    | Year 3    | Year 4    | Year 5    |
|-------------------------------------|-----------|-----------|-----------|-----------|
| F&A Cost Rate 1                     | 44.1%     | 44.1%     | 44.1%     | 44.1%     |
| F&A Cost Base 1                     | \$290,594 | \$276,094 | \$274,594 | \$270,694 |
| F&A Costs 1                         | \$128,152 | \$121,757 | \$121,096 | \$119,376 |

## A. COVER PAGE

|                                                                                                                                                                    |                                                                                                                                                                                                                                                   |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Project Title:</b> Understanding the Risk of Bat Coronavirus Emergence                                                                                          |                                                                                                                                                                                                                                                   |
| <b>Grant Number:</b> 5R01AI110964-02                                                                                                                               | <b>Project/Grant Period:</b> 06/01/2014 - 05/31/2019                                                                                                                                                                                              |
| <b>Reporting Period:</b> 06/01/2014 - 05/31/2015                                                                                                                   | <b>Requested Budget Period:</b> 06/01/2015 - 05/31/2016                                                                                                                                                                                           |
| <b>Report Term Frequency:</b> Annual                                                                                                                               | <b>Date Submitted:</b> 05/01/2015                                                                                                                                                                                                                 |
| <b>Program Director/Principal Investigator Information:</b><br>PETER DASZAK , PHD BS<br><b>Phone number:</b> (b) (6)<br><b>Email:</b> (b) (6)                      | <b>Recipient Organization:</b><br>ECOHEALTH ALLIANCE, INC.<br>ECOHEALTH ALLIANCE, INC.<br>460 W 34TH ST<br>17TH FLOOR<br>NEW YORK, NY 100012320<br><br><b>DUNS:</b> 077090066<br><b>EIN:</b> 1311726494A1<br><br><b>RECIPIENT ID:</b> 07-049-7012 |
| <b>Change of Contact PD/PI:</b> No                                                                                                                                 |                                                                                                                                                                                                                                                   |
| <b>Administrative Official:</b><br>ALEKSEI CHMURA<br>460 W 34th St., 17th Floor<br>New York, NY 10001<br><br><b>Phone number:</b> (b) (6)<br><b>Email:</b> (b) (6) | <b>Signing Official:</b><br>ALEKSEI CHMURA<br>460 W 34th St., 17th Floor<br>New York, NY 10001<br><br><b>Phone number:</b> (b) (6)<br><b>Email:</b> (b) (6)                                                                                       |
| <b>Human Subjects:</b> Yes<br>HS Exempt: No<br>Exemption Number:<br>Phase III Clinical Trial:                                                                      | <b>Vertebrate Animals:</b> Yes                                                                                                                                                                                                                    |
| <b>hESC:</b> No                                                                                                                                                    | <b>Inventions/Patents:</b> 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?

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### 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?

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

## 1) Conference and University lectures

- PI Daszak, and Co-investigators Olival and Shi gave >10 invited University lectures that included specific discussion of the current project and results.

## 2) Agency and other USG briefings

- NRC, 2015: Invited speaker, IOM Forum on public health preparedness, Interagency meeting on Medical Countermeasures. PI Daszak specifically reported on the findings from Year 1 of this project and the risk of SARS-like viruses causing future pandemics
- World Health Summit, Berlin 2014: PI Daszak was an invited panelist at a session on pandemic risk, and specifically reported the results and aims of this project
- International bat virus conference, Colorado, 2014: PI Daszak and Co-investigator Olival presented results from this study
- National Academies, Division of Earth & Life Studies, Spring Advisory Committee Meeting, DC. PI Daszak presented results from this study as part of an invited talk.
- Consortium of Universities for Global Health Conf., Washington DC, 2014. PI Daszak presented data from this study in a session on disease ecology

## 3) Public outreach

- PI Daszak reported on this project at an EcoHealth Alliance meeting hosted by the Cosmos Club, 2014

**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. Early in Year 2 of the study, it is anticipated that all of the qualitative research (i.e, 5-7 focus groups and ~100 ethnographic interviews) will be completed, transcribed and translated. It is anticipated that a total of approximately 100 ethnographic interviews and five to seven focus groups will be conducted in targeted areas with known bat populations in Yunnan, Guangxi, Guangdong and Fujian over the next few months. At least one of the focus groups and an estimated 35-40% of the interviews and surveys will be conducted with women. Subjects are enrolled in this study without regard to ethnicity.

Preliminary analyses will be conducted and will focus on the factors least understood, but crucial to the development of a behavioral risk survey that captures relevant behaviors and practices. Factors include specific human-animal interactions, experiences of unusual illness in both humans and animals, and an assessment of the context within which these activities occur. Because of the unique dataset and the expected richness of the data, additional research questions will be developed and explored using grounded theory, as well as more recently developed methods such as narrative analysis and case oriented understanding.

Results from preliminary analyses will contribute to the development of the behavioral risk survey. A behavioral survey sampling frame and recruitment materials are currently being developed. After pilot testing the behavioral survey, we will begin concurrent biologic specimen collection from bats, other wildlife and humans to compare circulating CoV strains in the bat population with serological exposure in human populations. The behavioral risk survey will facilitate the identification of explicit behavioral risks and practices that are found among study participants seropositive for SARS-like corona virus. These findings will be used to develop better risk mitigation policies and targeted intervention strategies.

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:

1. Parameterizing with actual data on species diversity and abundance of animals from Southern China markets.
2. Parameterizing with species-specific data on CoV prevalence and strain variation in different bat species from field surveillance, e.g. if *Rhinolopus* spp. represent the highest risk for SARS-related CoV emergence, these species will be given a higher weight.
3. Incorporation of CoV lineage specific probabilities for inter-host spillover based on receptor binding data.

We will also conduct further modeling activities, including:

1. 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.
  - a. Using previously published data from literature and Genbank
  - b. Using sequence data from our S. China surveillance
2. Calculate CoV divergence times using Spike RBD sequences for S. China.
3. Construct initial generalized linear mixed model to predict CoV diversity using S. China data and bat host-specific trait data. Update model regularly with new data from CoV screening in different bat species.

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

The following experiments will be undertaken in Year 2:

## 1. Animal infection experiment with SARS-like CoV

Option 1. Virus infection through ACE2 humanized mouse. Human ACE2 promotor (9-10 kb) and ACE2 will be inserted into a expressing vector and sent to a commercial company to generate transgenic mice. The stably expressed human ACE2 mice will be used for virus infection.

Option 2. Virus infection through SARS-CoV susceptible animals such as ferrets.

All above animal infection experiment will be performed under the containment of BSL3.

2. Continued surveillances of SARS-like CoVs in Yunnan and Guangdong provinces and isolation of novel virus strains.
3. Surveillance of infection in human populations by SARS-like CoVs. This work will be performed at two locations, one each in Yunnan and Guangdong provinces. PCR and ELISA will be used, respectively, for detection of viral replicase gene and antibody against the viral

nucleocapsid protein.

## Year 1 Report for Understanding the Risk of Bat Coronavirus Emergence

Award Number: 1R01AI110964-01

### B2: What was accomplished under these goals?

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

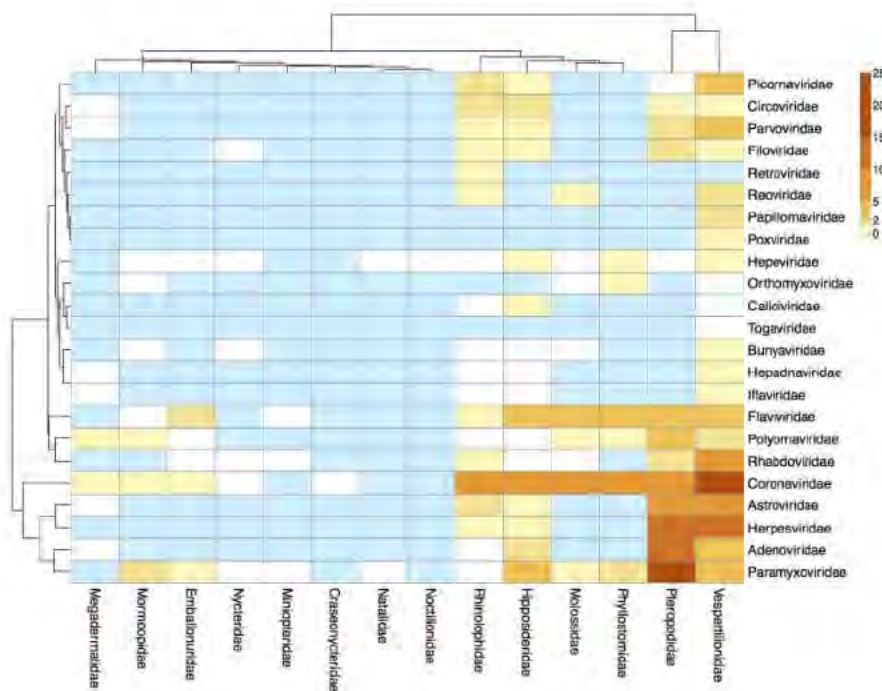
In the first year of this R01, we have:

- 1) Designed a behavioral risk study using an iterative approach that begins with rapid and focused qualitative research at or near biological surveillance sites in China where bats have previously been captured, sampled and found to contain novel CoVs. The study design includes: 1) structured observation and mapping of public spaces, 2) focus groups and 3) ethnographic interviews. The primary enrollment criteria are related to occupational exposure to bats and residence near bats. This research is conducted with two groups of individuals: those involved in the bat value chain (from hunter through market to consumer) and those highly exposed to bats (e.g., cave dwellers). The qualitative data will be used to inform a behavioral risk survey, as well as to contextualize findings from behavioral surveillance analyses.
- 2) Conducted observational research and mapping in: **Yunnan**: In and around Xiang Yun village (two clinics and one wildlife restaurant); in and around the remote Lu Feng village (1 wildlife farm, 1 wildlife butcher and 1 wildlife restaurant) and at the An Ning communicable disease hospital complex; **Guangxi**: In and around LiPu, (two markets, 3 wildlife farms, 1 wildlife restaurant); and **Guangdong**: Guangzhou wildlife market, Foshon wildlife market (this market is where the first cases of SARS were traced back to in 2003).
- 3) Secured local IRB approval in November 2014 from Wuhan University School of Public Health, Hubei Province, to conduct qualitative research, to administer behavioral surveys and to collect biological data including blood (no more than 550ml), sputum, and stool samples from humans. We secured US IRB approval through Hummingbird IRB (2014-23 approval letter sent to NIH) in November 2014 for qualitative, quantitative and biological specimen data collection.
- 4) Drafted protocols, guides, and training modules for Observational Research, Focus Groups, and Ethnographic Interviews and pilot tested these. The Observational Guide and Ethnographic Interview materials were pilot tested in live animal markets in Queens, New York City. Consistent with the original proposal, we have trained interviewers and identified key informants. Key informants include community health workers from three different administrative level CDCs, Barefoot Doctors, public health clinicians, local wildlife farmers and wildlife restaurant owners, as well as market vendors and workers. Ethnographic and Focus Group Interviews to be conducted pending NIH approval of IRB approval letter.

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

- 1) *Collation and preliminary analysis of published bat Coronavirus data to optimized specimen collection and taxonomic targets for surveillance.*

Over the last decade a large number of bat viral discovery studies have been published globally (including a large number focused on CoVs). In year 1, we conducted the first ever systematic analysis of these data. We collated literature from over 100 viral discovery studies in bats, to examine patterns of host range and known viral diversity in different bat taxa (Young and Olival, In Review). We found that Coronavirus diversity has been most thoroughly characterized in a few bat families, including the Vespertilionidae and 5 other families, but several bat taxa remain under-represented in global virus surveillance efforts (**Fig 1**). Identification of these surveillance gaps allows us to better target our field surveillance towards bat taxa where CoV diversity is largely unknown (blue and light colored cells, **Fig 1**). These analyses were completed at various taxonomic levels, including by bat subfamily and genera (Family level analysis only shown).



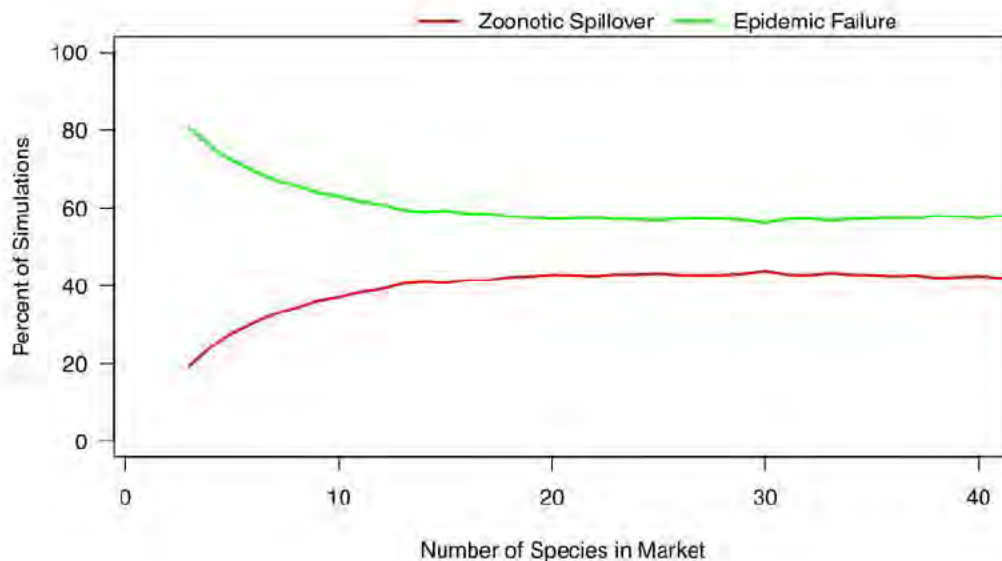
**Figure 1.** Heat map of viral richness by bat host and viral family, clustered by similarity in viral richness across host and viral families.

To maximize our chances of discovering CoVs, we need to define the number of specimens required for our bat surveillance work and the bat taxonomic groups on which to focus our surveillance. We used generalized linear mixed models (GLMM) and applied this to a subset of our collated data for CoVs alone. We found that sample type screened (feces), collection methods, and the number of specimens tested best explains the probability of finding an individual CoV positive sample. We will now use these

approaches to increase the likelihood of getting positive samples in our fieldwork in China.

- 2) *Preliminary 'What-if' Model: Role of species diversity in CoV emergence risk.* We built a mathematical model to analyze different scenarios of CoV spillover. We began with an assessment of how the diversity of wildlife (and other factors) in wet markets may affect the probability of CoV zoonotic spillover. We modeled evolution of CoVs within wildlife in a market following the initial introduction of a novel virus in one specific host. We assume this initial virus is a single genotype that does not yet have a great enough rate of spread to create an epidemic, but has a rate of spread close to this threshold. When this virus infects a new host, a new genotype is generated, based on random drift from the infecting genotype. We use Neutral Theory of Species Diversity to specify the species distribution in the market, for a given total number of species and total abundance of animals. We assume 500 animals in the market, and alter the species diversity from 3 to over 40. These numbers are easily attained in a small to medium market in Southern China (and in year 2 we will groundtruth these assumptions)

As the number of species present in a market increases from 3 to 20, the percent of simulations where zoonotic spillover occurred from any of the animals into humans increases (**Fig 2**). However, the risk remains fairly level if wildlife biodiversity increases above that level. The probability of epidemic failure is inverse to the probability of a zoonotic spillover taking hold and decreases with increasing species diversity (**Fig 2**). Therefore our null model shows that reducing the diversity of species in live animal markets could reduce the risk of zoonotic spillover, including of potentially pandemic CoVs.



**Figure 2.** 'What-if' scenario model based on the Neutral Theory of Species Diversity to examine the role of wildlife species diversity for CoV spillover in markets.

**Specific Aim 3: Testing predictions of CoV inter-species transmission.**1) *Bat Coronavirus Surveillance in 2014*

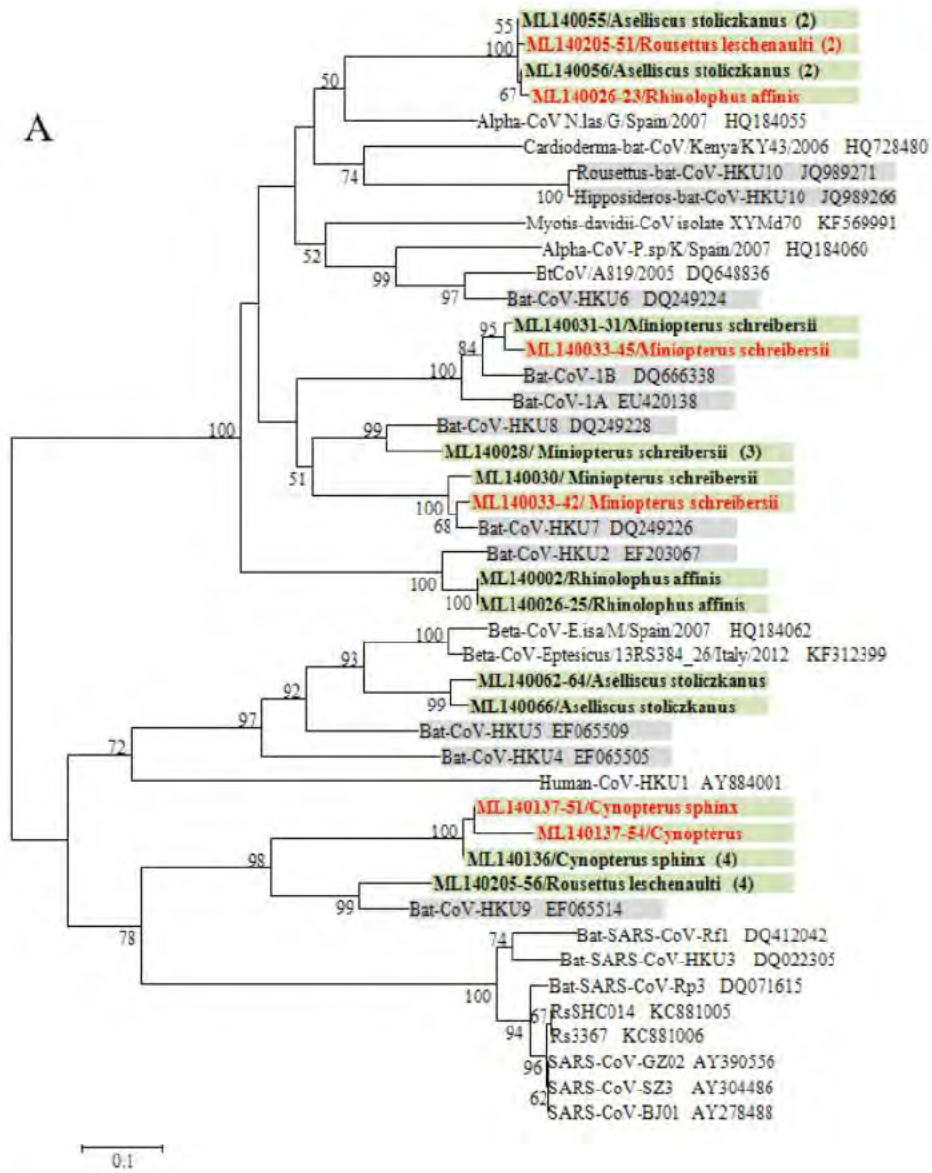
We collected 1555 anal swab samples, 1357 fecal samples, 461 blood samples, 469 serum samples and 24 tissue samples from > 14 bat genera in 5 provinces and in Laos (Table 1).

**Table 1** Bat Samples collected for CoV surveillance in 2014

|                 |                   | Anal | Oral | Fecal | Blood | Serum | tissue |
|-----------------|-------------------|------|------|-------|-------|-------|--------|
| Jan. 2014       | Mengla, Yunnan    | 164  | --   | --    | --    | --    | --     |
| Mar. 2014       | Beihai, Guangxi   | 30   | --   | --    | --    | --    | --     |
| April 2014      | Shenzhen          | 77   | --   | --    | --    | --    | --     |
| May 2014        | Ruyuan, Guangdong | 167  | --   | --    | --    | --    | --     |
|                 | Chuxiong, Yunnan  | 52   | 52   | 103   | --    | 8     | 16     |
|                 | Jinning, Yunnan   | --   | --   | 131   | --    | --    | --     |
|                 | Mojiang, Yunnan   | 25   | 25   | 103   | --    | --    | 3      |
| May-Sep. 2014   | Xianning, Hubei   | --   | --   | 583   | --    | --    | --     |
| Jun. 2014       | Guangdong         | 77   | --   | --    | --    | --    | --     |
| Jul. 2014       | Hainan            | 460  | --   | --    | --    | --    | --     |
| Aug. 2014       | Yichang, Hubei    | --   | --   | 114   | --    | --    | --     |
| Sep. 2014       | Guilin, Guangxi   | 121  | 122  | --    | 122   | 122   | --     |
|                 | Guangdong         | 335  | 337  | --    | 335   | 335   | --     |
| Jul.--Sep. 2014 | Mojiang, Yunan    | --   | --   | 96    | --    | --    | --     |
| Oct. 2014       | Jinning, Yunan    | 13   | 13   | 6     | 3     | 3     | 4      |

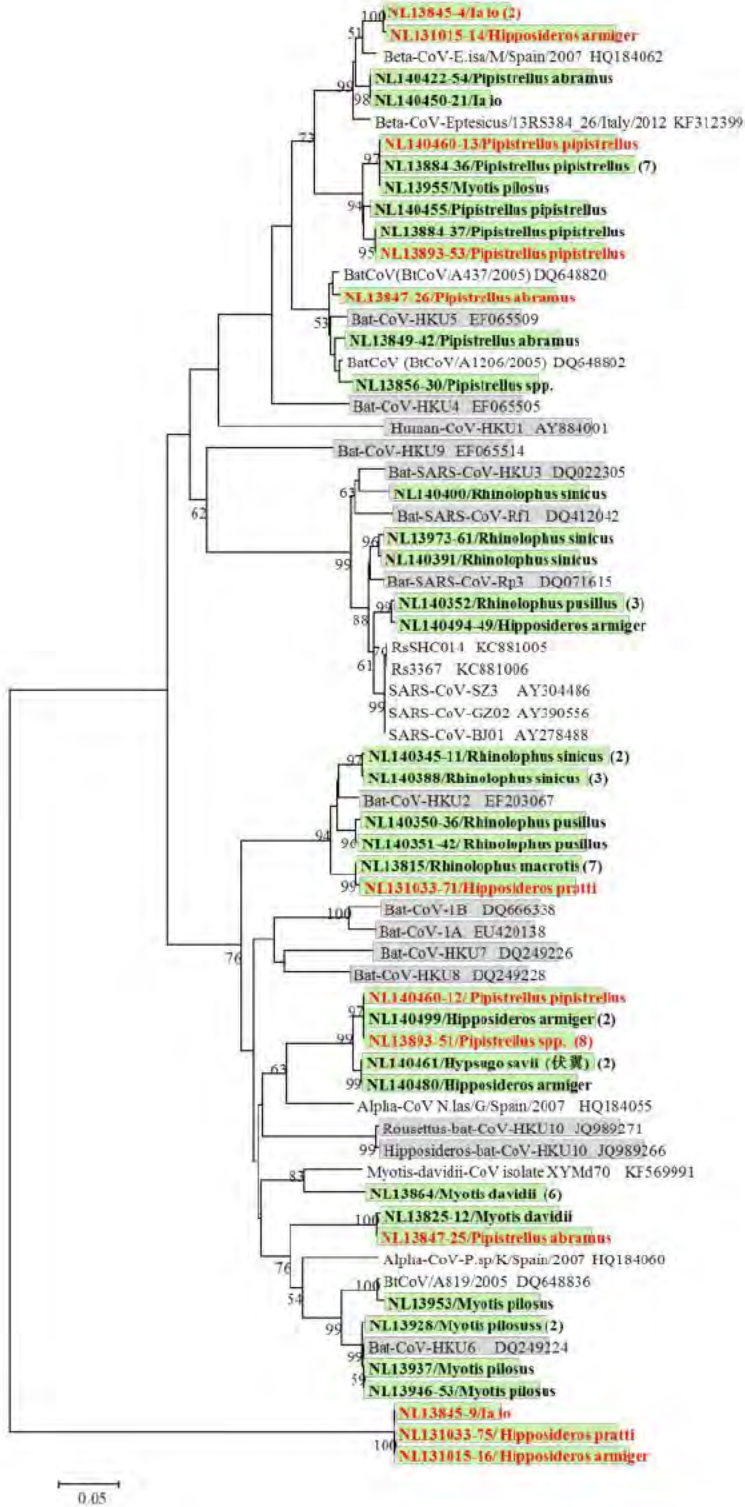
|  |                |      |     |      |     |     |    |
|--|----------------|------|-----|------|-----|-----|----|
|  | Mojiang, Yunan | 34   | 34  | 100  | 1   | 1   | 1  |
|  | Laos           |      |     | 121  |     |     |    |
|  | Total          | 1555 | 583 | 1357 | 461 | 469 | 24 |

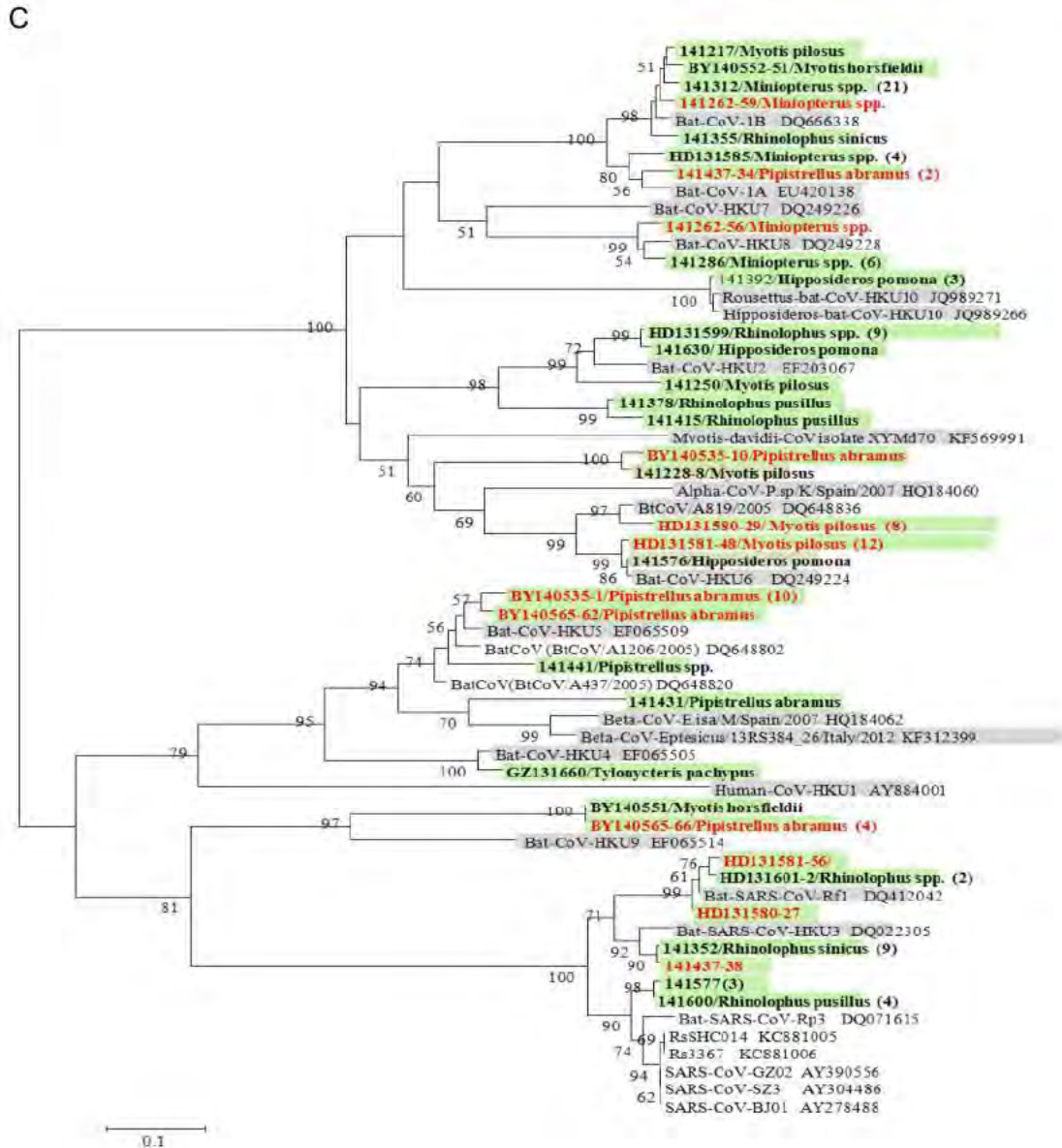
CoV was detected in 14% (336/2329) samples (**Table 2**). Diverse alphacoronaviruses were identified, including isolates closely related to Bat CoV 1A, 1B, HKU2, HKU6, HKU7, HKU8 and HKU10. Groups of novel alphacoronaviruses were discovered in a variety of bat species (**Fig 3**). **Novel SARS-like coronaviruses were detected in *Rhinolophus* bats collected in different regions of Guangdong province.** Diverse novel betacoronaviruses related to HKU5 were detected in *Pipistrellus* bats and *la io* in Guangdong and in *Aselliscus stoliczkanus* in Mengla, Yunnan. Novel coronaviruses related to HKU9 were found in *Cynopterus sphinx* and *Rousettus leschenaulti* in Mengla (**Fig 3A**). In addition, sequences significantly divergent to other CoV were obtained from three samples of *la io* and *Hipposideros* bats.





B





**Figure 3:** Phylogenetic analysis of partial RdRp gene of CoV. CoVs identified in this study are in bold and named by the sample numbers. Sequence amplified from samples co-infected with two CoV strains are indicated in red. (A) CoVs detected in Mengla, Yunnan. (B) CoVs detected in Ruyuan, Guangdong. (C) CoVs detected in other regions in Guangdong.

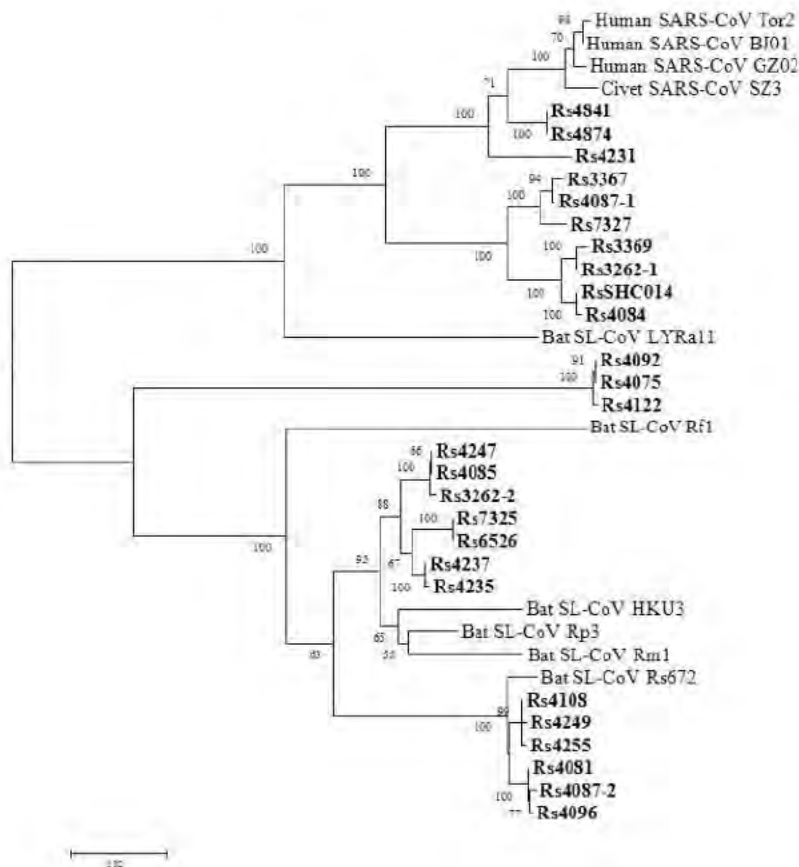
## 2) Complete S gene sequencing and recombination analysis of novel SARS-like CoV

We amplified the full-length S gene of the novel SL-CoV detected in a *Rhinolophus sinicus* colony in Yunnan Province. In addition to our previously reported Rs3367 and RsSHC014, we now have 24 new full-length S gene sequences from 22 samples.

Phylogenetic analysis showed that these SL-CoV are diverse, **and identified two strains of novel SL-CoV more closely related to SARS-CoV than Rs3367 (Fig 4A).**

Our new strains named Rs4841 and Rs4874 share the highest homology to SARS-CoV than any other known SL-CoV, including those we published previously in *Nature*.

These viruses are highly similar to SARS-CoV in receptor-binding domain (RBD) sequence but also in N-terminal domain (NTD) (**Figure 4B**). Analysis of the complete S protein shows > 97% amino acid identity to that of SARS-CoV isolates.



**Figure 4A**

Phylogenetic analysis of novel SL-CoVs discovered in Year 1 of this project (**Bold**), based on amino acid sequences of complete S gene.



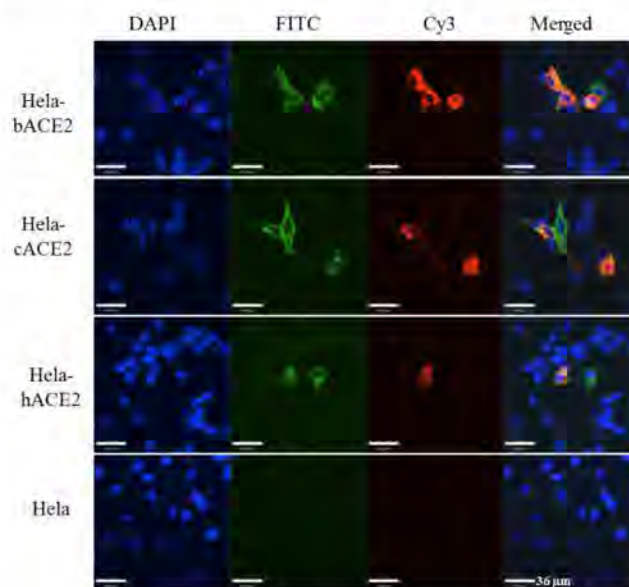
**Figure 4B** Alignment of amino acid sequences of S1 (aa1-680) of SARS-CoV and bat SL-CoVs.

We performed recombination analysis and detected potential recombination events in S genes of multiple SL-CoV strains suggesting that that the region around nt1000 in RBD is a recombination hotspot. In addition, a novel SL-CoV strain (Rs4075) with an NTD sequence distinct from all other SL-CoVs was identified (**Figure 4**). The results suggest that the high genetic diversity of SL-CoV in this colony is related to the frequent recombination.

### 3) *Virus isolation and characterization*

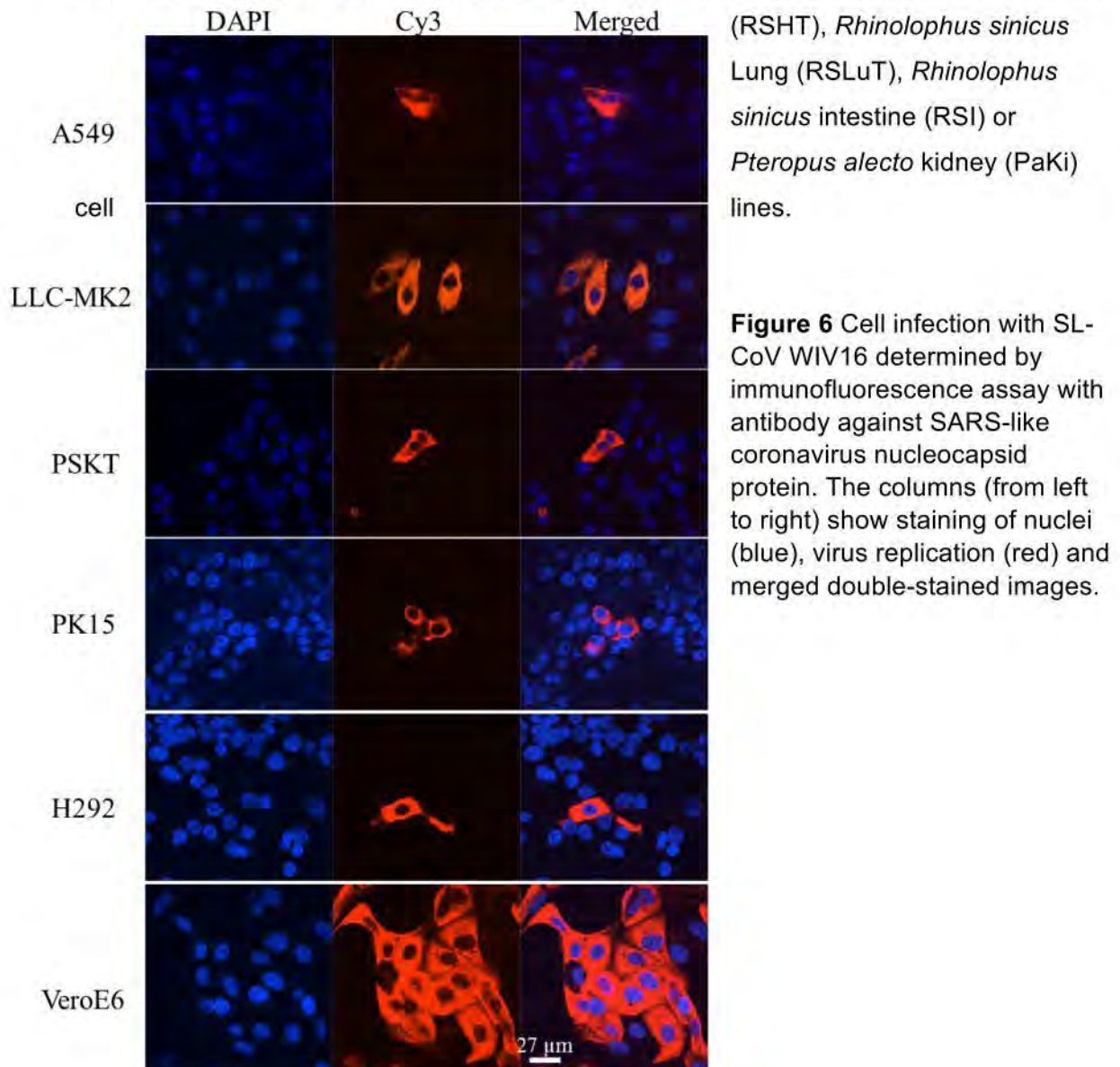
Isolation on Vero E6 cells was conducted on all CoV PCR-positive samples using an optimized protocol. Reproducible CPE was observed for Rs4841 (the strain closely related to SARS-CoV in both the RBD and NTD region of the S protein). Purified virions displayed typical coronavirus morphology under electron microscopy, and this novel isolate was named SL-CoV-WIV16.

We conducted virus infectivity studies (using HeLa cells expressing or not expressing ACE2 from humans, civets or Chinese horseshoe bats) to determine whether SL-CoV-WIV16 can use ACE2 as a cellular entry receptor (**Figure 5**). We found that WIV16 is able to use ACE2 of different origins as an entry receptor.



**Figure 5.** Analysis of receptor usage of SL-WIV16 determined by immunofluorescence assay. Determination of virus infectivity in Hela cells without the expression of ACE2. b, bat; c, civet; h, human. Nuclei are stained with DAPI. The columns (from left to right) show staining of nuclei (blue), ACE2 expression (green), virus replication (red) and merged triple-stained images.

To assess its cross-species transmission potential, we conducted infectivity assays in cell lines from a range of species. Our results (**Figure 6**) show that SL-CoV-WIV16 can grow in human alveolar basal epithelial (A549), pig kidney-15 (PK15), *Rhinolophus sinicus* kidney (RSKT), *Macaca mulatta* Kidney cell lines (MK2) and human lung carcinoma (NCI-H292), but not in human cervix (HeLa), Syrian golden hamster kidney (BHK21), *Myotis davidii* kidney (BK), *Myotis davidii* intestine (MDI), *Rousettus leschenaulti* kidney (RLK), *Rhinolophus sinicus* brain (RSBT), *Rhinolophus sinicus* heart



Daszak, Peter, PI

**Accomplishments for Understanding the Risk of Bat Coronavirus Emergence****Grant Number 5R01AI110964****B4: Opportunities for Training and Professional Development**

In year 1 of this work, we trained undergraduate interns from Columbia University in modeling approaches to understand bat risk of harboring zoonotic CoVs. In the behavioral risk work, we used standardized training materials for all three qualitative behavioral risk data collection methodologies have been created. Materials were used to train six people in New York City and 12 people in Yunnan, China, of which 11 were from three different administrative levels of local government Centers for Disease Control (CDC). The trainees include the Chinese EcoHealth Alliance Field Coordinator and Yunnan Provincial CDC personnel: six researchers from Xiangyun County CDC (4 women, 2 men), two from Yunnan Institute for Endemic Diseases (Yunnan Provincial CDC; 2 men), and three from Lu Feng County CDC (3 men).

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                                                                                                                                 |
|--------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| N/A: Not Journal         | Olival KJ, Weekley CC, Daszak P. Bats and Viruses. Wang L editor. New York: John Wiley & Sons, Inc.; 2015. What we know and need to know |
| Non-Compliant            | (b) (4)                                                                                                                                  |
| PMC Journal - In process | (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 |
|            | Y   | KE, CHANGWEN           |         |         | PHD             | Co-Investigator                |     |     |                  | CDC and Prevention of Guangdong Province                  | CHINA   | NA |
|            | Y   | ZHANG, YUNZHI          |         | (b) (6) | PHD             | Co-Investigator                |     |     |                  | Yunnan Institute of Endemic Diseases Control & Prevention | CHINA   | NA |
|            | Y   | ZHU, GUANGJIAN         |         | (b) (6) | PHD             | Co-Investigator                |     |     |                  | East China Normal University                              | CHINA   | NA |
| (b) (6)    | Y   | SHI, ZHENGLI           |         | (b) (6) | PhD             | Co-Investigator                |     |     |                  | Wuhan Institute of Virology                               | CHINA   | NA |
| (b) (6)    | N   | CHMURA, ALEKSEI A      | (b) (6) | (b) (6) | BS              | Non-Student Research Assistant |     |     |                  |                                                           |         | NA |
| (b) (6)    | Y   | OLIVAL, KEVIN J        | (b) (6) | (b) (6) | PHD             | Co-Investigator                |     |     |                  |                                                           |         | NA |
| (b) (6)    | Y   | HOSSEINI, PARVIEZ RANA | (b) (6) | (b) (6) | BS,PHD          | Co-Investigator                |     |     |                  |                                                           |         | NA |
| (b) (6)    | Y   | ZHANG, SHUYI           |         | (b) (6) | PHD             | Co-Investigator                |     |     |                  | East China Normal University                              | CHINA   | NA |
|            | Y   | GE, XINGYI             |         |         | PHD             | Co-Investigator                |     |     |                  | Wuhan Institute of Virology                               | CHINA   | NA |
| (b) (6)    | Y   | EPSTEIN, JONATHAN H    | (b) (6) | (b) (6) | MPH,DV M,BA,PHD | Co-Investigator                |     |     |                  |                                                           |         | NA |

**Glossary of acronyms:**  
 S/K - Senior/Key  
 DOB - Date of Birth  
 Cal - Person Months (Calendar)

Foreign Org - Foreign Organization Affiliation  
 SS - Supplement Support  
 RE - Reentry Supplement  
 DI - Diversity Supplement

Aca - Person Months (Academic)  
Sum - Person Months (Summer)

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?

No

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 |
|---------------|---------|
| 50902         | 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?**

Yes

As reported by Dr. Peter Daszak (PI) to NIH in May 2014, all of the following senior/key/other personnel were 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.

Peter Daszak, PI  
 Zhengli Shi, Co-Investigator  
 Shuyi Zhang, Co-Investigator  
 Changwen Ke, Co-Investigator  
 Jonathan Epstein, Co-Investigator  
 Kevin Olival, Co-Investigator  
 Parvies Hosseini, Co-Investigator  
 Xingyi Ge, Co-Investigator  
 Guangjian Zhu, Co-Investigator  
 Yunzhi Zhang, Co-Investigator  
 Aleksei Chmura, Program Coordinator

**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 District | Address                                                     |
|------------------------------------------|-----------|------------------------|-------------------------------------------------------------|
| <b>Primary:</b> EcoHealth Alliance, Inc. | 077090066 | NY-010                 | 460 West 34th Street<br>17th Floor<br>New York NY 100012317 |
| Wuhan Institute of Virology              | 529027474 |                        | Xiao Hong Shan, No. 44<br>Wuchang District<br>Wuhan         |
| East China Normal University             | 420945495 |                        | 3663 Zhongshan Beilu<br>Shanghai                            |

#### G.9 FOREIGN COMPONENT

**Organization Name:** East China Normal University

**Country:** CHINA

**Description of Foreign Component:**

Institution of Co-Investigators Dr. Shuyi Zhang and Dr. Guangjian Zhu

**Organization Name:** Wuhan Institute of Virology

**Country:** CHINA

**Description of Foreign Component:**

Primary Laboratory and Institute of Co-Investigators Dr. Zhengli Shi and Dr. Xingyi Ge

**Organization Name:** Yunnan Institute of Endemic Diseases Control and Prevention

**Country:** CHINA

**Description of Foreign Component:**

Institution of Co-Investigator Dr. Yunzhi Zhang

**Organization Name:** Center for Disease Control and Prevention of Guangdong

**Country:** CHINA

**Description of Foreign Component:**

Institution of Co-Investigator Dr. Changwen Ke

#### 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

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

**Foreign/Domestic:** Foreign

**Planned Enrollment Report**

**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 Report**

**NOTE:** No cumulative inclusion enrollment data exists in the previous inclusion format or modified format. Although prompted to do so, the PD/PI did not enter information in the modified format. No data can be provided.