## B. ACCOMPLISHMENTS

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

Zoonotic coronaviruses are a significant threat to global health, as demonstrated by the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoir of SARS-CoV-like viruses (including >260 by our group). These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a large-scale human-wildlife interface, and high risk of future emergence.
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 influenced by anthropogenic change. Specifically, we will assess the nature and frequency of contact among animals and people in markets in rural China. In the markets we hypothesize that high viral diversity in bats and other wildlife species, coupled with high contact rates between bats and people, will be associated with increased risk of CoV emergence. We will test this hypothesis by: 1) characterizing the diversity of CoVs in bats and other wildlife sold in markets in China and Southeast Asia; 2) assessing the risk of CoV emergence in humans by determining the frequency of contact between humans and bats and other wildlife in markets, and by identifying CoVs with the potential for human emergence; and 3) identifying the ecological and anthropogenic factors that drive CoV emergence. We will address these three specific aims and will screen free ranging and captive bats in China and Southeast Asia, and other wildlife sold in markets in China, for CoVs. We will also assess the risk of high-risk occupational exposure to bats and other wildlife in China and Southeast Asia. We have already described a large number of novel CoVs in bats in China and Southeast Asia, and have developed models to examine the risk of future bat-CoV spillover to humans. This work will be complemented by studies to examine the potential for bat-CoVs to emerge in China, and to identify the key risk factors for CoV emergence.

Specific Aim 1: Assessment of CoV spillover potential at high-risk human-animal interfaces in China. We will test the hypothesis that enhanced viral diversity in bats and other animals in China increases the likelihood that bat CoVs will infect other species, either via evolutionary change or recombination, and that the importation 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 in these markets; collect blood samples from people exposed to wildlife; and collect a full range of clinical samples from bats and other mammals in the markets.

Specific Aim 2: Receptor evolution, host range and predictive modeling of CoV emergence. We will test the hypotheses: 1) CoV host-range in bats and other mammals is limited by the phylogenetic relatedness of host species to the evolutionary conservation of CoV receptors; 2) CoV host-range is influenced by ecological opportunity for contact between species so that the wildlife trade disrupts the 'natural' co-phylogeography of CoVs and promotes viral evolution. We will develop CoV phylogenetic trees from sequence data collected previously by our group, and in the proposed study, as well as from Genbank. We will examine co-evolutionary congruence between CoVs and their hosts for receptor (receptor) and neutral genes. We will predict host-range in unsampled species using a generalized linear mixed 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 models to predict CoV [illegible] and to examine scenarios of how CoVs with different [illegible] dynamics will respond to [illegible].

Specific Aim 3: Identification of host range (i.e. emergence potential) of bat-CoVs. We will test the hypothesis that 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 [illegible] or expressing different receptor molecules [illegible] potential for each isolated virus and those with receptor binding [illegible] to spill over [illegible] from all our bat-CoV [illegible] mutants to identify how significantly each would [illegible] to use ACE2, CD26/DPP4 (MERS-CoV) [illegible] receptors. We will then use receptor-mutant pseudoviruses in [illegible] studies in bat, primate, human and other [illegible] lines, and with humanized mice where particularly interesting viruses are identified. We will use our predictive model to better target bat species and CoVs for public health-relevant data, and also iteratively refine our predictive model to better target bat species and CoVs for 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 approval of the project?

No

### B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Year 2 NIAID CoV Report Final.pdf

### B.3 COMPETITIVE [illegible]

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

No

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

File uploaded: Year 2 NIAID [illegible]oV Report Professional.pdf

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

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

2) Agency and other briefings: PI Daszak and Research Technician Guangjian Zhu introduced this project to [illegible] laboratory within the following agencies: Forestry Dept of Peoples' Republic of China, FAO, TNC, TRAFFIC, China CDC, and TA Foundation in Beijing, China. EcoHealth Alliance, the State Forestry Administration of China, and China CDC.

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

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

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

- Given the reduced amount of wildlife in the local markets within Southern China and the continued expansion of the Chinese wildlife trade within SE Asia, we would like to conduct short field trips to markets, but also sample species of bat and other high-risk hosts in countries that export wildlife to China (Myanmar, Laos, Vietnam) and countries that import wildlife for international trade to China (Thailand, Malaysia, Indonesia). EcoHealth Alliance has other activities in these countries which would provide leverage to reduce costs of fieldwork, and [illegible] be tested in Kunming, China.

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

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

- After the success of our pilot studies in Year 2, we will continue targeted surveillance of individuals with high behavioral and biological survey work in Yunnan and expand to Guangxi and Guangdong provinces.

- We will commence our anonymized passive surveillance data collection from [illegible] hospitals in Yunnan province. Eligibility criteria are: (a) suspected acute viral [illegible] infection and (b) presenting with symptoms of at least one of the following:
•Encephalitis of unknown origin
•Hemorrhagic fever of unknown origin
•Respiratory illness (SARI)
oSevere Acute Respiratory-like Illness (SARI)
•Rash
•Diarrhea
Some patients with pandemic [illegible] such as SARS. Our anonymized passive hospital surveillance allows for data collection and testing from all eligible hospital patients thereby limiting population sample bias and increasing the chances of identifying positive cases. The strengths of this approach are enormous: a large unbiased patient population; prospectively collected, anonymized patient data; a low resource effort with a high efficiency design; and impactful research potential for both case series and case control studies. We have already secured approval from the Institutional Review Boards of the Wuhan University School of Public Health and Hummingbird IRB.

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

- Testing and refinement our response-driven survey to collect specific data on bat diversity, abundance, and [illegible] in the wildlife trade network in southern China.

- Model viral mixing across the full range parameters found along the wildlife trade network to identify the trade nodes with highest mixing potential. This will include [illegible] along the wildlife trade network to identify the trade nodes with highest mixing potential. This will include [illegible] operations, transit hold[illegible] wildlife markets.

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

China.

- Phylogeo[illegible] populations and infer their historical mo[illegible]ments a[illegible]nd [illegible]
Preliminary sequences data has been generated and will be completed and analyz[illegible]

- Cophylog[illegible]
and Beta-CoV co[illegible]phylogen[illegible]ic patterns building on Year 2 analyses using published sequences and also includ[illegible]
additional sequences obtained in Year 2.

[illegible]
network.

- Examine co-evolutiona[illegible]ence of bat-CoVs and their hosts using both functional (receptor) and neutral genes;

- Parameterize mathematical models that predict Co[illegible]iary and transmission dynamics
[illegible] surveillances of SARS-like CoVs and lineage C betacoronaviruses (MERS-r[illegible]

- Full-length genome [illegible] and evolution analysis of SARS-like coronaviruses identified from different bat species at different geographical l[illegible]

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

- Full-length genome sequencing and evolution analysis of HKU9-rela[illegible]d and HKU10-related bat c[illegible]

Specific Aim 3: Testing p[illegible]a[illegible]

- Humanized mice with huma[illegible] ACE[illegible]
determine the tissue tropism and pathogenicity of bat SL-CoV

- Isol[illegible] novel ba[illegible]
receptor [illegible]olecules. Spillover potential for each isolated virus will be assessed.

- An infectious clon[illegible] full-length MERS-C[illegible]
MERS-related viruses identified from [illegible]inese bats, the chimeric viruses with S gene of bat MERS-related coronaviruses and backbone of the [illegible]ous clone of MERS-CoV will be construc[illegible]ed to study the receptor usage and infectivity of b[illegible]RS-related

- Surveillance of in[illegible]n populations by SARS-like CoVs. This work will be performed at locations in Yunnan, Guangxi, a[illegible]
Guangdong provinces, in[illegible]eviously [illegible]ied areas with human popula[illegible]
[illegible]spectively, for detec[illegible] gene and antibodies a[illegible]

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**Year 1 Report:** Understanding the Risk of Bat Coronavirus Emergence

**Award Number:** 1R01AI110964-03

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**Section B: Accomplishments**

**B.1 What are the Major Goals of the Project**

Zoonotic ... 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 ... group as bat species, and since then hundreds of novel bat-CoVs have been discovered (>260 by our group). These, and other wildlife species, are hunted, traded, butchered ... 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, 2) the ... evolutionary potential, and how it might force CoV evolution. We will assess the nature and frequency of contact among animals and people ... two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In these ... we hypothesize 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 the ... specific aims and will screen free ranging and captive bats in China for known and novel ... screen people who have occupational exposure to bats and other wildlife ... and examine ... properties of novel bat-CoVs we have already identified ... these we will discover. We will then use ecological and evolutionary analyses and predictive mathematical ... examine the risk of future bat ...

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. We will examine if: 1) wildlife ... either via evolutionary adaptation or recombination ... Southeast Asia introduces a higher genetic diversity ... 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 ... highly exposed to wildlife; and collect a full ... of clinical ... from ... in the wild and in wetmarkets; and screen these for CoVs using serological and molecular assays.

Specific Aim 2: Receptor evolution, host range and ... models of ... CoV emergence risk. We propose two competing hypotheses: 1) CoV host-range in bats and ... 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 ... disrupts the natural co-phylogenetic ... facilitates spillover and ... CoV phylogenies from sequence data collected previously by our group, and in the proposed study, as well as from Genbank. We will examine ... 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 ... 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: Predictions of CoV host species transmission and spillover risk. We will assess the range (i.e. emergence potential) experimentally using reverse genetic, pseudovirus and receptor binding assays, and virus isolation in cell culture. Using the sequences of CoVs that we've isolated or sequenced, and using live virus or pseudovirus infection of cells of different origin or expressing different receptor molecules, we will assess potential for variation in the 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-[illegible] and other species' cell lines, and with humanized mice where molecularly interesting viruses are identified phylogenetically, or isolated. These tests will provide public health relevant data, and also iteratively improve our predictive models, informing our field studies to obtain bat CoV strains of the greatest relevance to human health, and the most informative to understand the mechanisms of cross-species transmission.

**B.1a Have the major goals of the project been achieved? [illegible]**

**B.2 What was accomplished under these goals?**

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

In year 2 a community based integrated biological behavioral surveillance system was developed and pilot tested to identify specific animal exposures associated with biological evidence of exposure to SARS-like CoV (i.e., seropositive status).

**QUALITATIVE RESEARCH**

Targeted, in-depth ethnographic interviews were conducted with rural Southern China where wildlife trade risks have been documented. Yunnan, Guangxi and Guangdong provinces were specifically selected for study because they have a high diversity of wildlife species and numerous live animal markets. Yunnan and Guangxi provinces border Southeast Asia, a region of global importance for the emergence of novel zoonotic diseases. In-depth interviews were conducted in Yunnan province at nine different sites, 24 (51%) in Guangxi province at six different sites. In addition, one focus group was conducted in Guangdong. The study was approved by the Institutional Review Boards of the Wuhan School of Public Health and Hummingbird IRB.

Participant recruitment sites in each province included [illegible] wildlife restaurants, live animal markets, caves where people do tourism or collect guano and residential areas/farms near known bat caves or roosts. Participants were recruited primarily through local contacts developed as part of wildlife conservation and [illegible] past decade. Contacts including wildlife traders, outreach workers and wildlife farmers facilitated introductions and provided referrals. To obtain a sample with sufficient representation of categories of interest, participants were recruited using

purposive [illegible] which provides minimum quotas in terms [illegible]
(e.g., live animal market, forest [illegible])

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

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

| Genus species | Common Name |
|---|---|
| *Prionailurus bengalensis* | Leopard |
| *Nyctereutes procyonoides* | Raccoon Dog |
| *Sus scrofa* | Wild Boar |
| *Lepus sinensis* | Chinese Hare |
| *Arctonyx collaris* | Hog Badger |
| *Hystrix brachyura* | Porcupine |
| *Marmota sp.* | Marmot |
| *Rhizomys 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 |
| *Cervus* | Sambar Deer |
| *Ovis aries* | Sheep |
| *Capra sp.* | Domestic Goat |
| *Ratus norvegicus* | Common Rat |

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

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

Markets in Guangzhou found wildlife to be plentiful (see Table 1), although no bats were seen for sale during the observation period.

In contrast, wildlife was not found in live animal markets in 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 it less visible than income generating [illegible]

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A [illegible] experience in the past 12 months were evaluated. Of those with symptoms, 75% reported: raising animals, animals in the home, buying live animals; 50% reported [illegible] (75%) reported handling/preparing recently killed animals, 11 (69%) Handling live animals or having animals in the home, [illegible] reported slaughtering, 9 (56%) raised live animals, 7 (44%) reported a pet, and 1 (6%) reported animal feces near food or eating animal touched e[illegible] damaged food, hunting, or eating raw/undercooked animal products). Finally, among the [illegible] raising animals, 2 (50%) reported animals in the home, 1 (25%) reported having animals as pets, slaughtering/killing animals, or having bought live animals at wet market.

Respondents were asked about the sou[illegible] exposure as a potential source of infection and most stated they had no idea how they had become infected. However, when asked about potential behavior changes made at live animal markets in the last 12 months, participants reported a great deal of change. In particular, respondents reported buying live animals less often (38%), only buying farmed wildlife (54%), and no longer buying wildlife at wet markets (23%). (See Table 3).

**Table 3: Behavior Change at Wet M[illegible]**

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

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

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

Nucleic acid test results of human biological samples

*Testing High-Risk Human Populations for Coronavirus Infection*

Year 2, including both custom-built ELISA serology (an assay developed by the Wuhan Institute of Virology to test antibodies against the N protein of SL-CoV) and PCR detection of viral RNA.

*Serological test for SL-CoV antibodies in human samples from [illegible] Province*

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

*PCR test for CoV Nucleic Acid in human samples from several Provinces*

We collected 405 [illegible] populations and to obtain sequence data on strain variation, individual samples (4 each) were pooled prior to nucleic acid extraction then tested using PCR. When a group tested positive, we then conducted the confirmation test in the individual samples. One single sample (14XN611) from someone who had identified as having had a fever a [illegible] identified to be positive for [illegible] unexpected. [illegible] will be improved in Year 3 by better targeting of [illegible] individuals for specimen collection and continuing to optimize PCR assays. Refined serological assays (above) will provide sufficient data to assess past exposure to specific CoV lineages, and optimizing of PCR detections will allow for more CoV positive [illegible] sequences for [illegible]

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

*Bat CoV PCR detection and sequencing from live-sampled bat populations*

We collected 1,714 anal swab samples, [illegible] fecal samples, 53 blood samples and 38 serum samples from 15 bat genera in Guangdong, Yunnan, Sichuan, Hubei, Hunan, Guizhou, Guangxi provinces [illegible]

**Table 4 Bat Samples collected for CoV surveillance**

| Sample date | Sample location | Anal | Fecal | Blood | Serum |
|---|---|---|---|---|---|
| Mar. 2015 | Huidong, Guangdong | 69 | -- | -- | -- |
| Jun. 2015 | Guangdong | 495 | -- | 12 | |
| Apr. 2015 | Mengla, Yunnan | 91 | -- | -- | |
| May 2015 | Jinning, Yunnan | -- | 193 | -- | -- |
| May. 2015 | Mojiang, Yunnan | 93 | -- | -- | -- |
| Oct. 2015 | Jinning, Yunnan | 30 | -- | -- | -- |

**Table 5 Test result of bat CoV surveillance in 2015 – 12% positi**

| | Yunnan | Guangdong | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Bat species** | | | | | | | | |
| *Rhinolophus spp.* | 47/98 | 12/103 | | | | 16/225 | 8/63 | 83/489 |
| *Hipposideros spp.* | 0/35 | 0/ | | | | | | |
| *Ia io* | | | | | | 0/3 | | 0/3 |
| *Pipistrellus spp.* | 1/1 | 0/19 | | | | 0/2 | 0/4 | 1/26 |
| *Miniopterus spp.* | 6/ | | | | | | | |
| *Eonycteris spp.* | 0/3 | | | | | | | 0/3 |
| *Vespertilio superans* | | | | 41/128 | 41/128 | | | 41/128 |
| *Myotis spp.* | | 1/38 | | | | 0/70 | 0/35 | 1/143 |
| *Taphozous spp.* | 0/25 | | | | | 0/1 | | 0/26 |
| *Tynalycteris pachypus* | | 8/25 | | | 27/191 | | | 35/216 |
| *Scotophilus kuhlii* | | 1/1 | | | | | | 1/1 |
| *Eptesicus fuscus* | | 0/1 | | | | | | 0/1 |
| *Tadrida spp.* | | 0/5 | | | | | | 0/5 |
| *Barbastella* | | | | | | | 0/1 | 0/1 |
| *Nyclatus velutiaus* | | | | | | | 0/10 | 0/10 |
| Fecal samples | 28/468 | | 22 | 180 | | | | 50/648 |
| **Sub-total** | 82/637 | 56/326 | 48/332 | 41/128 | 27/191 | 16/438 | 8/204 | 280/2256 |

colored circle), 14 host switches (solid colored circle with arrow), 0 losses

Our findings demonstrate co-speciation alone is not sufficient to explain the observed co-phylogeny pattern and several host switches occurred specifically identified [illegible] global signal of co-speciation has been detected. This work highlights, the need for these types of detailed coph[illegible]

*References cited in the above analysis:* Agnarsson, [illegible] Torrello, C.M., Flores-Sa[illegible] May-Co[illegible] [illegible] [illegible]rated species-level phylogen[illegible] (Chiroptera, Mamma[illegible] *Current Biology*, 5:RRN1212. Asher, R.J. & Hergett, M.J. (2010) Nomenclature and placental mammal phylogeny. *BMC Evolutionary Biology*, 10, 1-9. Lei BR, Olival KJ (2014) Contrasting Patterns in Mammal–Bacteria Coevolution: *Bartonella* and *Leptospira* in Bats and Rodents. *PLoS Negl Trop Dis* 8(3): e2738.

*Market Characterization Model Parameterization*

Our ongoing observational research and mapping of farms and [illegible] trade. The nexus of the wildlife trade and the potential hotspots of interspecies viral mixing is now in many cases in animal storage facilities and transport between high-volume customers. To define realistic parameters for intermixing wildlife species in areas of high potential mixing, we have developed a preliminary survey and sampling protocol to assess these [illegible] through these storage facilities - using [illegible] and sample along the wildlife trade network and reveal hidden nodes and sites of int[illegible] of species.

We have expanded our intermixing modeling framewo[illegible] chain, where the diversity, abundan[illegible] residence time, and contact rates [illegible] animals move through the trade network.

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

In Year 2, we continued surveillance for novel SARS-like [illegible] Yunnan and Guangdong provinces and obtained full genome sequence for 11 CoV [illegible] isolates was completed, including phylogenetic and recombination analyses. Importantly, recombination analysis of the full-length SL-CoV genome sequences from a single bat population revealed that frequent recombination events among different SL-CoV strains occur. Several SL-CoVs that are more genetically similar to SA[illegible] (2003) than any previously discovered were also identified [illegible] Yunnan provi[illegible] genome analysis [illegible] and p[illegible] more insight in[illegible]

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

To date, including preliminary data submitted for this R01 that we are now analyzing under the curre[illegible] funding, we have conducted 5-years of surveillance of SL-CoV in a single bat colony in Yunnan Province (from 2011 to 2015), leading to the discovery of diverse novel SL-CoVs by the region corresponding to the receptor binding domain (RBD) of SARS-CoVs. 11 isolates were [illegible] in Year 2.

These SL-CoVs, including four others isolated previously from this colo[illegible] 5367, Rs3hC014, WIV1 and WIV16, are highly diversified in the S gene, but share similar sequence identity to SARS-CoV in the [illegible] (Fig 4). Genomic phylogenetic analysis showed [illegible] CoVs detected in this colony are more [illegible]

*Additional Year 2 items for Specific Aim 2:*

- The infectious clone of WIV1 was successfully constructed using reverse genetic methods,
- Two chimeric bat SARS-like coronavirus strains were constructed by replacing the S gene in the bac
- Permission to import mice with huma experimental infec

- Comparative cophylogenetic analyses of bat host and CoV RdRp and Spike gene phylogenies assess patterns of evolutionary congruence and frequency of cross-species transmission (This will be conducted in ye
- Ani fection experiments of S coronaviruses were n done, because of the begin this wo in year 3.
- Sampling of bat and other mammalian species in markets to screen for CoVs. We will begin this work in year 3.

Section C: Accom lications

PUBLISHED

Xing-Yi Ge, Ning Wang, Wei Zhang, Bo Hu, Boping Yang, Zhi Zhang, Xing-Lo Yang, Li-Jun Wu, Bo Wang, Yun Zhang, Zheng-Xiao Li, and Zheng-Li Shi. Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft. *Virologica Sinica* 31, 31 (2016).

Mei-Niang Wang, Wei Zhang, Yu-Tao Gao, Bo Hu, Xing-Yi Ge, Xing-Lou Yang, Zhi Zhang, Zheng-Li Shi. Longitudinal surveillance of SARS-like C *Sinica* 31(1): 78-80 (2016).

Cristin C. W. Yo

Kevin J. Olival. To C To Cull Bat is the Ques

Xing-Lou Y g, Ben Hu, Bo Wang, Mei-Niang W Zhi Zhang, Peter Daszak, Jin-Fa Wang, Zheng-Li Shi. Isolation and characterization coronavirus closely related to the ancestor of severe acute respiratory syndrome coronavirus, *Journal of V* (90(6): 3253-6 (2015).

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

ACCEPTED, IN PRESS

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

## B.4 WHAT OPPORTUNITIES FOR TR[illegible]

**B.4 What opportun[illegible]**

We presented our project to grad[illegible] Hospitals in Yun[illegible] (YNCDC), Dali Provincial Hospital, and The Third People's Hospital of Kunming. Select doctors at YNCDC (1) and Dali Provincial Hospital (3) were tra[illegible]

We trained gra[illegible] Public Health (3) in qualitative behavioral risk data collection meth[illegible] technologies, survey dat[illegible] S[illegible] the University of Miami (http://citiprogram[illegible] education conten[illegible]

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ZHENGLI | | | | In[illegible] | | | | of Virology | | |

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization
SS - Supplemental Support
RE - Reentry Supplement
DI - Diversity Supplement

**D.2 PERSONNEL UPDATE**

**D.2.a Level of Effort**

**Will there be, in the [illegible] budget period, either (1) a reduction for the PD/PI(s) or other senior/key personnel designated in the Notice of Award minimum amount of effort required by the Notice of Award?**

No

**D.2.b New Senior/Key Personnel**

**Are there, or will there be, new senior/Key personnel?**

Yes

File uploaded: Noam Ross CV 2016.pdf

**D.2.c Changes in Other Support**

**Has there been a change 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 [illegible]**

NA

## AWARDS + FELLOWSHIPS (*Total received $225,420*)

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

## SERVICE + PROJECTS

- **Workshop Instructor,** Software Carpentry and Data Carpentry
- **Student Rep,** UC Davis Graduate Group in Ecology Executive Committee
- **Reviewer:** *Theoretical Ecology*
- **Web Developer and Technology Chair,** Ecology Graduate Student Association
  *Creator + Maintainer of graduate student*
- **Founder + Organizer,** Davis R Users' Group Sep 2012–Present
  *Created users group that provides tutoring and seminars to graduate students in statistical computing*
- **Contributor,** R packages knitr, knitcitations, rcrossref, rethinking 2012–Present
- **Organizer: NSF REACH IGERT Workshop on Multiple Goals in Floodplain**
- **Organizer,** UC Davis Conference on Ecology and the Business Sector Apr 2011
- **Organizer,** UC Davis Graduate Group in Ecology Symposium May 2010-2011
- **External Reviewer**
- **Grant Reviewer,** McKnight Foundation Global Initiative Project 2009
- **Business Stewardship Volunteer,** NY Coastal Marine Research Center

## OTHER WORK EXPERIENCE

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

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

**Wal-Mart** Providence, RI
*Contract Researcher/Consultant* Jan 2006–Aug 2006

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

## E. IMPACT

### E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF THE HUMAN RESOURCE AND INSTITUTIONAL CAPABILITY?

Not Applicable

### E.2 WHAT IS THE IMPACT ON THE DEVELOPMENT OF THE INFRASTRUCTURE THAT SUPPORTS RESEARCH?

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 COUNTRIES OTHER THAN THE UNITED STATES?

| | Country |
|---|---|
| 211[illegible]9 | CHINA |

## F. CHANGES

### F.1 CHANGES IN APPROACH AND REASONS

Not Applicable

### F.2

NOTHING TO REPORT

### F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS

#### 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 OPPORTUNITY ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

### G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

### G.3 MENTORING

Not Applicable

### G.4 HUMAN SUBJECTS

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

Yes

**Is the research exempt from HHS regulations?**

No

**Does this project involve a clinical trial?**

No

### G.4.b Inclusion Enrollment Data

Report Attached: Guidance to Risk of Bias & Coronavirus Emergence-PROTOCOL-001

### G.4.c Clinical Trials.gov

**Does this project involve a clinical trial?**

No

### G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

**Are there individuals conducting clinical research or conducting**

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 District |
|---|---|---|

| | | District | |
|---|---|---|---|
| Primary: EcoHealth Alliance, Inc. | 077090066 | NY-010 | 460 West 34th Street<br>17th Floor<br>New York NY 100012317 |
| Wuhan Institute of Virology | 529027474 | | Wuchang District<br>Wuhan |
| East China Normal University | 420945495 | | |
| ECOHEALTH ALLIANCE | 07709 | | NEW YORK NY 100012320 |
| 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 North Road<br>Shanghai |

**G.9 FOREIGN COMPONENT**

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

**Organization Name:** East China Normal University
**Country:** CHINA
**Description of Foreign Component:**
Principal Collaborating Team for all project field work as per section G8 (above) and

**G.10 ESTIMATED UNOBLIGATED BALANCE**

**G.10.a Is it anticipated that an estimated unobligated balance (funds remaining at the end of the budget period) will be greater than 25% of the budget period'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 Data Set or Resource?: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Foreign

NIH Defined Phase II

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

## Planned Enrollment

**Planned Enrollment Total:** 2,460

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

## Cumulative Enrollment

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