



Additional information follows















B. ACCOMPLISHMENTS

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

Zoonotic coronavirus (CoV) outbreaks are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV-1999) and Middle East Respiratory Syndrome (MERS-CoV) in wildlife reservoirs (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 zoonotic CoV emergence. To understand the risk of zoonotic CoV emergence, we are examining 1) the transmission dynamics of bat CoVs across the human-wildlife interface, and 2) how this process is affected by human activities. We will assess the nature and frequency of contact among animals in China and people who are involved in wildlife trade, and the potential for CoV spread by heightened mixing of host species leading to viral evolution, and high potential for CoV spread among three specific aims and will screen free ranging and captive bats in China for high 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 wildlife and collect a full range of clinical samples from bats and other mammals in the region.

Specific Aim 1: Assessment of CoV spillover potential at high risk areas in China. We will enhance surveillance for CoVs to infect other 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 wildlife and collect a full range of clinical samples from bats and other mammals in the region.

Specific Aim 2: Receptor evolution, host range and predictive modeling. Hypotheses: 1) CoV host-range in bats and other mammals is limited by the phylogenetic relatedness of host species so that the wildlife trade disrupts the natural co-phylogeny and promotes viral evolution. We will develop CoV receptor genes from sequence data collected previously by our group, and in the proposed study, as well as from GenBank. We will examine co-evolutionary congruence (receptor) and neutral genes. We will predict host-range in unsampled species using a generalizable model of host range based on phylogenetic traits to explain patterns of viral sharing between species. We will use data to parameterize a model of cross-species transmission and use data to parameterize a model of cross-species transmission.

experimentally using reverse genetics, pseudovirus and receptor binding domain (RBD) CoVs, and virus infection experiments in cell culture and humanized mice. With bat-CoVs that we have identified, we will identify the potential for each isolated virus and those with receptor binding domain (RBD) sequences to spill over to humans. We will use receptor-binding domain (RBD) sequences from all our bat-CoVs to generate mutants to identify how significantly each would differ from the wild type. We will use ACE2, CD26/ DPP4 (MERS-CoV receptor) and other receptors. We will use receptor-mutant pseudovirus to test for cross-species transmission in bat, human and other species. We will use public health-relevant data, and also interview people about wildlife trade to better target our 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 proposal?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE ADVANTAGES OF THIS PROJECT?

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?

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

- 1) **Conferences and University lectures:** PI Daszak and Co-investigators Shi, Jin, Ojiva, 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 Biomedicine, 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 Guojunian Zhu introduced this project to 200+ laboratory and field workers within the following agencies: Forestry Dept of Peoples Republic of China, FAO, INR, TRAFFIC, China CDC, and IA Foundation in Beijing, EcoHealth Alliance, the State Forestry Administration of China, and China Wildlife Conservation Association.
- 3) **Public outreach:** PI Daszak and Co-investigator Zhu presented this project at 100+ public meetings including: EcoHealth Alliance meeting hosted by the Cosmos Club, Washington D.C. (2015); PI Daszak and Co-investigator Zhu presented on this project at a Wildlife Trade and Public Health Seminar in Beijing (2016); PI Daszak introduced this project in a lecture at the New York Academy of Sciences (2016); Co-investigator Zhang presented project and results to date to department heads and senior researchers at Infectious Disease Department of the University of Michigan (2015).

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

**Specific Aim 1: Assessment of wildlife spillover potential at high risk nodes within the wildlife trade network in Southeast Asia.**

- Given the reduced amount of wildlife in the local markets within Southeast China and the continued expansion of the Chinese wildlife trade within SE Asia, we would like to conduct short-term field studies to assess wildlife trade in other high-risk hosts in countries such as Thailand, Malaysia, and Indonesia. EcoHealth Alliance will conduct field activities in these countries which would provide leverage to reduce costs of fieldwork, and to build capacity for wildlife trade monitoring and control.

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

- We will develop and conduct a pilot collection tool for a network study of wildlife farmers using a questionnaire to characterize and map the wildlife value chain.

- After the successful completion of our pilot studies in Year 2, we will continue to target individuals with high risk of wildlife trade in behavioral and biological survey work in Yunnan and expand to Guangxi and Guangdong provinces.

- We will commence our anonymized surveillance data collection in Year 3.

- Eligibility criteria are: (a) suspected acute viral illness
- Encephalitis of unknown origin
- Hemorrhagic fever of unknown origin
- Respiratory illness
- Severe Acute Respiratory like Illness (SARI)
- Rash
- Diarrhea

Some patients with potential for wildlife trade surveillance has the advantage of being hospitalized. Anonymized, passive hospital surveillance allows for data collection and surveillance using an eligible population of hospitalized patients thereby limiting population sample bias and identifying positive cases. The strengths of this design are enormous: a) unbiased population, b) prospectively collected, anonymized patient data; a low resource effort with a high efficiency design; and impactful research with potential for both case series and case control studies. We have already secured approval from the Institutional Review Boards of the Wildlife School of Public Health and Hummingbird IRB.

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

Future steps to optimize the model of role of species diversity in CoV emergence risk will include:

- Test the model using our response surface survey to collect specific data on wildlife diversity and trade in the wildlife trade network in Southeast Asia.
- Model viral mixing across the full range parameters found along the wildlife trade network to identify the trade nodes with highest mixing potential: transit hubs, border crossings, and wildlife markets.
- Phylogeographic study of bat-CoV to better understand the geographic distribution and evolution of bat-CoV genetic diversity in south

China.

- Phylogenetic relationships among bat-CoV populations and infer their historical movements and dispersal patterns building on Year 2 analyses using published sequences and also including preliminary sequences data that has been generated and will be completed and analyzed in Year 2.

- Cophylogenetic relationships between bat-CoV and Beta-CoV cophylogenetic patterns building on Year 2 analyses using published sequences and also including additional sequences obtained in Year 2.

network.

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

- Parameterize mathematical models that predict CoV primary and transmission dynamics

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

- Full-length genome sequencing and evolution analysis of Lineage C bat coronavirus (MERS-related) from different geographical locations across China.

- Full-length genome sequencing and evolution analysis of HKU9-related and HKU10-related bat coronavirus.

Specific Aim 2: Testing bat coronavirus receptor

- Humanized mice with human ACE2 receptor to determine the tissue tropism and pathogenicity of bat SL-CoV

- Isolation of novel bat coronavirus receptor molecules. Spillover potential for each isolated virus will be assessed.

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

- Surveillance of bat coronavirus populations by SARS-like CoVs. This work will be performed at locations in Yunnan, Guangxi, and Guangdong provinces, in previously unexplored areas with human populations. Samples will be collected, respectively, for detection of S gene and antibodies against S protein.

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Year 1 Report: Understanding the Risk of Bat Coronavirus Emergence

Award Number: 1R01AI110964-01

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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 of Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified as bat species, and since then hundreds of novel bat-CoVs have been discovered by our group. These, and other wildlife species, are hunted, traded, butchered in Asia, creating a large-scale 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 at two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In the process, we hypothesize that 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 following specific aims and will screen free-ranging and captive bats in China for known and novel bat-CoVs; we will examine occupational exposure to bats and other wildlife, and examine the genetic diversity and 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 emergence.

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. We will examine if: 1) wildlife reservoirs of CoVs are either via evolutionary adaptation or recombination; 2) 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 animal samples from both the wild and in wet markets, and screen them for CoVs using serological and molecular assays.

Specific Aim 2: Receptor evolution, host range and predictive modeling of CoV emergence risk. We propose two competing hypotheses. 1) CoV host-range in bats and other mammals is limited by phylogenetic relatedness of bats and evolutionary conservation of CoV receptors; 2) CoV host-range is limited by geographic and ecological opportunity. We will reconstruct bat-CoV phylogenies from sequence data collected previously by our group, and in the proposed study as well as from GenBank. We will examine the evolution of CoV receptors, 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 factors. We will test for positive selection in market samples.

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 spread through wildlife markets.

#### Specific Aims and Predictions of CoV

range (i.e. emergence potential) experimentally using reverse genetic approaches; pseudovirus and non-receptor binding assays, and virus infection of cell culture systems.

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

receptor binding site sequence, to pull in over we will do this by generating libraries of different binding/fusion) protein genes from all known 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

and other specific lines and with highly purified proteins identified phylogenetically, or isolated. These tests will provide public health relevant data, and also

iteratively improve our predictive models, refining our field studies to

obtain high quality strains of the ground truth, and using our field studies to

transmission.

#### B.1a Have the major

#### 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 (e.g., seropositive status).

#### QUANTITATIVE RESEARCH

Targeted, in-depth ethnographic interviews were conducted with rural Southern China where wildlife trade has been documented. Yunnan, Guangxi and Guangdong provinces were specifically selected for study because they have high diversity of wildlife species and numerous live animal markets.

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. All research was approved by the Institutional Review Boards of the Wunan School of Public Health and Nanjing University.

Recruitment sites in each province included wildlife restaurants, live animal markets, caves where people do bat or collect eggs and residential areas/farms near known bat caves or roosts. Participants were recruited primarily through local contacts developed as part of wildlife conservation activities in the past decade. Contacts including wildlife conservation outreach workers and wildlife farmers facilitated introductions and provided referrals. To ensure sample with sufficient representation of categories of interest, participants were recruited using

purposive sampling which provides minimum quotas in terms of location (e.g., live animal market, forest).

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

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

Genus species	Common Name
<i>Prionailurus bengalensis</i>	Leopard
<i>Nyctereutes procyonoides</i>	Raccoon Dog
<i>Sus scrofa</i>	Wild Boar
<i>Lepus sinensis</i>	Chinese Hare
<i>Arctonyx collaris</i>	Hog Badger
<i>Hystrix brachyura</i>	Porcupine
<i>Marmota sp.</i>	Marmot
<i>Rhizomys sinensis</i>	Bamboo Rat
<i>Erinaceus sp.</i>	Hedgehog
<i>Mustela putorius</i>	Ferrets
<i>Muridae</i>	Rat (species unknown)
<i>Myocastor coypus</i>	Nutria
<i>Vulpes sp.</i>	Fox
<i>Mustela sibirica</i>	Siberian weasel
<i>Paguma larvata</i>	Masked Palm Civet
<i>Felis catus</i>	Domestic Cat
<i>Canis lupus familiaris</i>	Domestic Dog
<i>Cervus mandchuricus</i>	Siamoa Deer
<i>Ovis arcticus</i>	Wild Goat
<i>Capra hircus</i>	Domestic Goat
<i>Rattus norvegicus</i>	Common Rat

Interviews were conducted between March and June 2015 by 10 interviewers, none of whom had prior experience with wildlife.

Interviews were conducted between one and 22 interviews; the interviewers conducted two-thirds of all interviews. Interviews lasted between 20 and 60 minutes, and were recorded and transcribed verbatim before they were translated into English. All participants received cooking oil valued at US\$10 in appreciation of their participation.

The data are currently being coded and an analytic database is being developed. Insights from the study suggest that 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 roads and renovate local infrastructure with the intention of increasing tourism. This has reduced forested area.

Wetmarkets 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 profitable. It is possible that income-generating activities have replaced wildlife hunting.



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experience, 13 (100%) reported symptoms, 75% reported: raising animals, animals in the home, buying live animals; 50% reported (75%) reported handling/preparing recently raised animals, 11 (69%) Handling live animals or having animals in the home, 10 (69%) reported slaughtering, 9 (56%) raised live animals, 7 (47%) reported a pet, and 1 (6%) reported animal feces near food or eating animal touched damaged food, hunting, or eating raw, undercooked, animal products. Finally, among the 100% of participants who reported animals in the home, 1 (25%) reported having animals at a petting zoo, slaughtering/killing animals, or having bought live animals at wet market.

Respondents were asked about the source of their exposure as a potential source of infection and reported 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 (23%). (See Table 3).

**Table 3. Behavior Change at Wet Market**

Behavior	N	(%)
Wear a mask	4	(3.0)
Wear gloves	5	(3.8)
Wash hands	5	(3.8)
Sometimes shop for meat at supermarket	30	(22.7)
Buy live animals less often	53	(37.9)
Buy only farmed wildlife	71	(51.8)
No longer buy wildlife at wet market	35	(25.0)

The results of this pilot study conducted in a region with a high level of unusual illness, as well as high levels of exposure to animals. There was a notable lack of knowledge of animals' ability to transmit infection. Despite this lack of knowledge, 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 a mobile application for use on either a mobile device or computer. Electronic data collection will facilitate data collection. Four field team leads were trained on behavioral survey data collection technologies (the table application) on an arbitrary basis.

**Nucleic acid test results of human biological samples**

*Testing High-Risk Human Populations for Coronavirus*



Year 2, including both virus-specific 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 viraemia.

*Serology test for SL-CoV antibodies in human samples from bat habitats*

In order to assess past exposure to bat CoV, 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 readings and neutralization titres to WIV1 and WIV16 was interpreted. These findings are encouraging; however, no neutralization antibodies were detected. In Year 3, we will continue to validate and optimize these ELISA assays and other serological assays for SL-CoV exposure.

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

We collected 405 human samples from bat habitats in order to assess past exposure to bat CoV in the population 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, they then conducted the confirmation test in the individual samples. One single sample (14XN611) from someone who had identified as having had a fever was identified as having had a fever. This finding was unexpected. In Year 3, we will continue 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 detection in sequence.

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

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

We collected 1,714 anal swab samples, 575 fecal samples, 53 blood samples, and 38 serum samples from 15 bat genera in Guangdong, Yunnan, Sichuan, Hubei, Hunan, Guizhou, Guangxi provinces.

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

Sample date	Sample location	Anal	Fecal	Blood	Serum
Mar. 2015	Huidong, Guangdong	68	--	--	--
Jun. 2015	Guangdong	495	78	12	--
Apr. 2015	Mengzi, Yunnan	51	84	--	--
May 2015	Jinming, Yunnan	--	103	--	--
May. 2015	Mojiang, Yunnan	79	--	--	--
Oct. 2015	Jinning, Yunnan	30	30	--	--



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

Bat species	Yunnan	Guangdong	North					
<i>Rhinolophus spp.</i>	17/98	12/103				16/225	2/63	23/489
<i>Hipposideros spp.</i>	0/35	0/1						
<i>Ia io</i>						0/3		0/38
<i>Pipistrellus spp.</i>	1/1	0/19				0/2	0/4	1/26
<i>Miniopterus spp.</i>	0/1							
<i>Eonycteris sp.</i>	0/3							0/3
<i>Vespertilio superans</i>			11/128	11/128				11/128
<i>Myotis spp.</i>		1/38				0/70	0/35	1/143
<i>Taphozous spp.</i>	0/25					0/1		0/26
<i>Tynolactis pachypus</i>		8/25		27/191				35/216
<i>Scotophilus kuhlii</i>		1/1						1/1
<i>Eptesicus fuscus</i>		0/1						0/1
<i>Tadrida spp.</i>		0/5						0/5
<i>Barbastella</i>							0/1	0/1
<i>Nyctalus velutius</i>							0/10	0/10
Fecal samples	28/168		27/180					50/648
<b>Sub-total</b>	<b>82/637</b>	<b>55/326</b>	<b>48/332</b>	<b>41/128</b>	<b>27/191</b>	<b>18/438</b>	<b>8/204</b>	<b>280/2256</b>





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

Our findings demonstrate CoS speciation alone is not sufficient to explain the observed CoV phylogenetic pattern and several host switches are specifically identifiable. Although a strong global signal of co-speciation has been detected, this work highlights the need for these types of detailed copy

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 Coevolution of *Bartonella* and *Leptospira* in Bats and Rodents. *Am J Trop Dis* 8(3): e2738.

#### Market Characterization Model Parameterization

Our ongoing observational research and mapping of farms and 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-density customers. To define key limiting parameters for intermixing wildlife species in areas of high potential mixing, we have developed a preliminary survey and sampling protocol to assess these facilities. We will survey and sample along the wildlife trade network and reveal hidden nodes and sites of interspecies

#### We have expanded our intermixing modeling framework

chain, where the diversity, abundance, residence time, and contact rates of 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 viruses in Yunnan and Guizhou provinces and obtained full genome sequence for 11 CoV isolates. This genome sequencing project was completed, including phylogenetic and recombination analyses. Importantly, recombination analysis of the full-length SL-CoV genome sequences from a single bat population revealed that frequent recombination events among different SL-CoV strains occur. Several SL-CoVs that are more genetically similar to SARS-CoV-2 were identified in Yunnan province. This genome analysis and phylogenetic analysis more insight into the evolution of SARS-CoV-2.

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

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

These SL-CoVs, including four others isolated previously from this colony (R3507, R3510C014, WIV1 and WIV16), are highly diversified in the S gene, but share similar sequence identity to SARS-CoV isolates (Fig 4). Genomic phylogenetic analysis showed that the CoVs detected in this colony are more









Additional Year 2 items for Specific Aim 2

- The infectious clone of WIV1 was successfully constructed using reverse genetic methods,
- Two C57BL/6J mice were infected with WIV1 using intranasal and intraperitoneal routes. Mice were monitored for clinical signs and mortality.
- Permission to import mice with human coronavirus HKU1 for experimental infection was granted.

- Comparative cophylogenetic analyses of bat host and CoV RdRp and S gene phylogenies will assess patterns of evolutionary congruence and frequency of cross-species transmission. This work will be conducted in year 2.
- Animal infection experiments of SARS-like coronaviruses were not done, because of the unavailability of suitable animal models. These experiments will begin this winter.
- Sampling of bat and other mammalian species in markets to screen for CoVs. We will begin this work in year 3.

Section C: Accomplishments

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Xing-Yi Ge, Ning Wang, Ben Hu, Peter Daszak, Zhi Zhang, Zheng-Li Shi, and Xing-Lou Yang. Coexistence of multiple coronaviruses in several bat species in an abandoned mine shaft. *Virology* 514(2): 249-254 (2016).

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Xing-Lou Yang, Ben Hu, Bo Wang, Mei-Niang Wang, Zhi Zhang, Peter Daszak, Lin Fa Wang, and Zheng-Li Shi. Isolation and characterization of a novel coronavirus related to the bat coronavirus HKU1 from a Chinese civet. *Journal of Virology* 90(6): 3253-6 (2015).

Ben Hu, Xingyi Ge, Linfa Wang, Zhi Shi, Peter Daszak, and Zheng-Li Shi. A novel coronavirus associated with a fatal case of acute respiratory distress syndrome in China. *Virology* 471(1): 221 (2015).

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Lei-Ping Zeng, Yu-Tao Gao, Xing-Yi Ge, Qian Zhang, Ben Hu, Peter Daszak, and Zheng-Li Shi. The SARS-like coronavirus WIV1 encodes an extra accessory protein ORF8 in addition to ORF1-4, which is involved in modulation of the host immune response. *Journal of Virology* 90(11): 5011-5018 (2016).

## B.4 WHAT OPPORTUNITIES FOR

### B.4 What opportunities

We presented our project to grade 12 students at Yunnan Provincial Hospital (YNCH), Yunnan Provincial Hospital (YNCH), and the Third People's Hospital of Kunming. Selected doctors at YNCH (1) and Yunnan Provincial Hospital (3) were trained.

We trained grade 12 students at Yunnan Provincial Hospital (1) and Yunnan Provincial Hospital (3) in qualitative behavioral risk data collection and survey data analysis. The training was conducted by the University of Miami (<http://citirprogram.org>) and the education center.





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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  
 SS - Supplemental Support  
 RE - Recurrent Support  
 DI - Diversity Supplement

**D.2 PERSONNEL UPDATE**

**D.2.a Senior/Key Personnel**

Will there be, in the current budget period, either (1) a reduction in the number of senior/key personnel designated in the Notice of Award or (2) a reduction in the minimum amount of effort required by the Notice of Award?

No

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

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

Yes

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

Has there been a change in other support 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 MPI leadership?

NA



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

- Don DeSoto 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 (\$115,000) UC Davis, CA, 2010  
*A world of change*
- UC Davis Graduate Group in Ecology Fellowship (\$40,604) UC Davis, CA, 2010
- NSF Research Experiences 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 Dependency and Data Curation
- **Student Rep.**, UC Davis Graduate Group in Ecology
- **Reviewer: Theoretical Ecology**
- **Web Developer**, UC Davis Graduate Group in Ecology  
*Creator + Maintainer of graduate student website*
- **Founder + Organizer**, UC Davis R Users Group  
*Created users group that provides tutorials and documentation to students*
- **Contributor**, R packages: *shinr*, *kintr*, *crossiter*, *retinr*
- **Organizer**, NSF REACH IGERT Workshop on Multiple Goals in Ecosystems
- **Organizer**, UC Davis Conference on Ecology and the Business Sector
- **Organizer**, UC Davis Graduate Group in Ecology Symposium
- **External Reviewer**
- **Peer Reviewer**, *Making a Better World: Global Issues in Ecology*
- **Business Stewardship Volunteer**, RI Coastal Marine Resources Center

**OTHER WORK EXPERIENCE**

**GreenOrder**

*Analyst, Senior Analyst: Corporate Environmental Strategy, Governance*

- Conducted environmental performance analysis for products in energy, transportation, and water sectors
- Created green product metrics from life stage gating system for construction products
- Managed engagement with external company to identify green opportunities in green building
- Performed market and competitive analysis, with an emphasis in clean tech sectors; prepared and delivered client presentations, managed projects
- Managed analysts performing environmental impact studies
- Developed firm seminar series and analyst training materials; conducted trainings on topics including auditing, statistical analysis, and environmental impact
- Audited certifications for environmental products and facility performance

**Wal-Mart**

*Contract Researcher/Consultant*

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





E. IMPACT

E.1 WHAT IS THE IM

Not applicable

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT

2110000

CHINA

F. CHANGES

F.1 CHANGES

Not Applicable

F.2

NOTHING TO REPORT

F.3 SIGNIFICANT

F.3.a Human

F.3.b Vertebrate Animals

No Change

F.3.c Biohazard

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

**G.1 SPECIAL REPORT**  
 NOTHING TO REPORT

**G.2 RESPONSIBLE CONDUCT**  
 Not Applicable

**G.3 MEN**  
 Not Applicable

**G.4 HUMAN SUBJECTS**  
**G.4.a Does the project involve human subjects?**  
 Yes  
**Is the research an experiment?**  
 No  
**Does this project involve a clinical trial?**  
 No

**G.4.b Inclusion Enrollment Data**  
 Report Attached

**G.4.c Clinical Trials.gov**  
 Does this project include...

**G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT**  
 Are there... or conduct...

**G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)**  
 Does this project involve human embryonic stem cells (only hESC lines listed and approved in the NIH Registry may be used in NIH funds for research)?

**G.7 VERTEBRATE ANIMALS**  
 Does this project involve vertebrate animals?  
 Yes

**G.8 PROJECT/PERFORMANCE SITE**

Organization Name	FUNDS	Congress District
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		District	
Primary: EcoHealth Alliance, Inc.	0177090066	NY-010	460 West 34th Street 17th Floor New York NY 100012317
Wuhan Institute of Virology	529027474		Wuchang District Wuhan
East China Normal University	420945		
EcoHealth Alliance	077090066		NEW YORK NY 100012320
EcoHealth Alliance, inc.	077090066	NY-010	460 West 34th Street 17th Floor New York NY 100012317
Wuhan Institute of Virology	529027474		Ying Huan Street No. 44 Wuchang Wuhan
East China Normal University	420945		3000 Zhongyuan Road Shanghai

G.9 FOREIGN COMPONENT

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

**Organization Name:** East China Normal University  
**Country:** CHINA  
**Description of Foreign Component:**  
 Principal Investigator's research team for an 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 (over) will be greater than 25% of the year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the performance period?

No

G.12 F&A COSTS

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

No

