## A. COVER PAGE

| | |
|---|---|
| **Project Title:** Understanding the Risk of Bat Coronavirus Emergence | |

| | |
|---|---|
| **Grant Number:** 5R01AI110964-05 | **Project/Grant Period:** 06/01/2014 - 05/31/2019 |
| **Reporting Period:** 06/01/2017 - 05/31/2018 | **Requested Budget Period:** 06/01/2018 - 05/31/2019 |
| **Report Term Frequency:** Annual | **Date Submitted:** 09/16/2020 |
| **Program Director/Principal Investigator Information:**<br><br>PETER DASZAK , PHD BS<br><br>**Phone number:** (b) (6)<br>**Email:** (b) (6) | **Recipient Organization:**<br><br>ECOHEALTH ALLIANCE, INC.<br>ECOHEALTH ALLIANCE, INC.<br>460 W 34TH ST<br>17TH FLOOR<br>NEW YORK, NY 100012320<br><br>**DUNS:** 077090066<br>**EIN:** 1311726494A1<br><br>**RECIPIENT ID:** NIAID Coronavirus |
| **Change of Contact PD/PI:** N/A | |
| **Administrative Official:**<br><br>ALEKSEI CHMURA<br>460 W 34th St., 17th Floor<br>New York, NY 10001<br><br>**Phone number:** (b) (6)<br>**Email:** (b) (6) | **Signing Official:**<br><br>ALEKSEI CHMURA<br>460 W 34th St., 17th Floor<br>New York, NY 10001<br><br>**Phone number:** (b) (6)<br>**Email:** (b) (6) |
| **Human Subjects:** Yes<br>HS Exempt: No<br>Exemption Number:<br>Phase III Clinical Trial: | **Vertebrate Animals:** Yes |
| **hESC:** No | **Inventions/Patents:** No |

## B. ACCOMPLISHMENTS

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

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

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

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

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

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

No

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

File uploaded: Year 4 NIAID CoV Report_Final for eRA Commons.pdf

### B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

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

No

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

File uploaded: Year 4 NIAID CoV Training and Prof Devlp.pdf

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

1.Conference and University Lectures: PI Daszak, and Co-investigators Shi, Epstein, Olival, and Zhang gave invited University and Conference lectures including Harvard Univ. Columbia Univ., Tufts Univ., Mt. Sinai, the 2nd International Symposium on Emerging Viral Disease in China, the 2nd International Symposium on the Infectious Diseases of Bats in Colorado, Cell Symposia: Emerging and Re-emerging Viruses 2017 in Virginia, The International Union of Microbiological Societies 2017 National Academy of Sciences in Singapore, 2018 Borneo Quality of Life Conference in Malaysia, 2017 Chemical and Biological Defense Science and Technology (CBD S&T) in California, Prince Mahidol Award Conference in Bangkok, Collaboration for Environmental Evidence Meeting in Paris, US-China NSF Ecology and Evolution of Infectious Disease (EEID) Meeting, and others that included specific discussion of the current project and results.

2.Agency and other briefings: PI Daszak and Co-investigator Shi introduced this project and discussed new opportunities about predicting and preventing zoonoses within National Institute of Allergy and Infectious Disease Office, Defense Advanced Research Projects Agency, National Natural Science Foundation of China, Chinese Center for Disease Control and Prevention, US NASEM Forum on Microbial Threats, Chinese Academy of Sciences, and the Health Working Group at the US Embassy in Beijing.

3.Public outreach: PI Daszak and Co-investigator Shi, Epstein, Olival, have presented this work to the general public in a series of meetings over Year 4 including at Cosmos Club briefings that EcoHealth Alliances hosts in Washington DC, over 10 meetings on the China National Virome Project and the Global Virome Project in China, Europe, Australia, Southeast Asia and Latin America. Co-investigator Olival presented this work at a public event on Disease Transmission and Technologies in New York, co-investigator Ross presented this work at EcoHealth Webinar on wildlife trade network research. Zhu broadly introduced this work to the conservation and ecological research community in China through field training workshops.

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

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

•To commence an in-depth analysis of data collected from the integrated biological behavioral surveillance from Yunnan, Guangxi, and Guangdong provinces, incorporating questionnaires and serological testing results.
•To initiate lab analysis of human samples collected from the passive hospital surveillance from four hospitals in Yunnan province: 1) Dali College Affiliated Hospital; 2) Dali Prefecture Hospital; 3) Kunming No. 3 People's Hospital, and 4) Chuxiong Prefecture Hospital. The goal will be to identify examples of CoV spillover events in China that may lead to illness.

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

•To repeat and continue in vivo experiments of SARSr-CoVs with spike variants on hACE-expressing transgenic mice (survival rate, histopathological analysis, etc) to evaluate the risk of cross-species infection of different SARSr-CoVs to humans;
•Continue searching for the receptor of SARSr-CoVs with deletions in the homologous region of SARS-CoV RBD (i.e. Rp3, Rs672), and SARSr-CoVs that are unable to utilize bat ACE2 (e.g. Rs4231).
•Continue the phylogeographic study of bat-CoV with newly collected samples to better understand the geographic distribution and evolution of bat-CoV genetic diversity in south China and SE Asia.

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

•Using the full-length infectious cDNA clone of MERS-CoV, chimeric viruses with the spikes of newly identified MERSr-CoVs will be constructed. The pathogenesis of these MERSr-CoVs will be tested on the human DPP4-expressing mouse model that has already been developed and validated in Y4.
•To conduct a population genetics study of Rhinolophus sinicus ACE2s, including the amplification of ACE2 genes from R. sinicus samples of different origin, test of the usage efficiency of R. sinicus ACE2s of different origins by SL-CoVs and kinetics study on the binding of SL-CoV RBD to different R. sinicus ACE2s.
•In collaboration with South China Agrricultural University, gather data on the spatial structure and barn-level mortality records to parameterize our mathematical model of virus spread that incorporates a meta-population structure in individual and use this to fit the model on a training set of farms and validate it on a hold-out set.
•Using the intra-farm transmission model, we will (a) determine the characteristics of a farm that determine the likelihood and size of an outbreak given a spillover event, and (b) determine whether SADS and PEDV outbreaks on farms can be distinguished by differing dynamics, as measured by transmission parameters in our intra-farm transmission model.

1R01AI110964 Year 4 Report                                                     PI: Daszak, Peter

**Year 4 Report:** Understanding the Risk of Bat Coronavirus Emergence

**Award Number:** R01AI110964-03

**Reporting Period**: 06/01/2017 – 05/31/2018

\*\*\*\*\*\*\*\*\*\*\*\*\*

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

## Summary

The results of the 4th year of our R01 work are detailed below. They include:
- Completed behavioral risk survey questionnaires and biological sample data collection for 1,585 people in Yunnan, Guangxi, and Guangdong provinces.
- Preliminary analysis of behavioral survey responses exploring key risk factors relating to potential viral zoonotic disease spillover in China, indicating notable differences among the respondents in Guangdong, Guangxi, and Yunnan.
- Completed serologic testing of collected human samples for MERS-CoV, SARSr-CoV, HKU9 CoV and HKU10 CoV, showing the serologic evidence of spillover of bat SARS-related CoVs (7 people in Yunnan province) and HKU9 CoV (2 people in Guangxi province).
- Testing of samples from 671 individual bats to identify diverse alpha- and beta-coronaviruses.
- Genetic diversity and genomic characterization of beta-coronaviruses in fruit bats and characterization of the full-length genome sequence of a novel HKU9-related CoV.
- Analysis of host-virus phylogeography for all bat CoV RdRp sequences collected by our group in China from 2008-2015 (Alpha-CoVs: n = 491; Beta-CoVs: n = 326) to identify the geographic areas that are likely sources of origin/diversity for this important group of viruses.
- Identification of two novel MERS-related CoVs that use DPP4 receptor.
- *In vivo* infection of SARSr-CoVs with variants of S protein in human ACE2 (hACE2) expressing mice.
- Identification of a novel bat-origin CoV (swine acute diarrhea syndrome coronavirus, SADS-CoV) causing a multi-farm outbreak of fatal acute diarrhea in piglets in Guangdong (published in *Nature* in April 2018).
- Development of an intra-farm transmission model to understand SADS-CoV spread and help predict and prevent future outbreaks.

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

During Year 4 we completed behavioral risk surveys and biological sample collection from people at selected sites in three provinces in southern China (Guangdong, Guangxi, and Yunnan) and began analyzing the results.

1

1R01AI110964 Year 4 Report                                        PI: Daszak, Peter

## Behavioral Survey

We administered 1,585 surveys in Guangdong, Guangxi, and Yunnan provinces. Questions explored respondent health-seeking behavior, experiences with unusual illnesses, contact with wildlife and livestock, and general background information. Blood samples were collected from respondents and tested for SAS-related CoVs (SARSr-CoVs) and HKU10-CoV using serological assays. Survey data was analyzed by province to examine patterns among respondent characteristics and behavioral risk factors across provinces.

## Respondent General Background Information

Of the 1,585 respondents who completed the survey, 420 were from Guangdong, 412 were from Guangxi, and 753 were from Yunnan. More females than males completed the survey in all provinces. The mean age of the overall survey sample was 52 years (**Figs. 1, 2**).
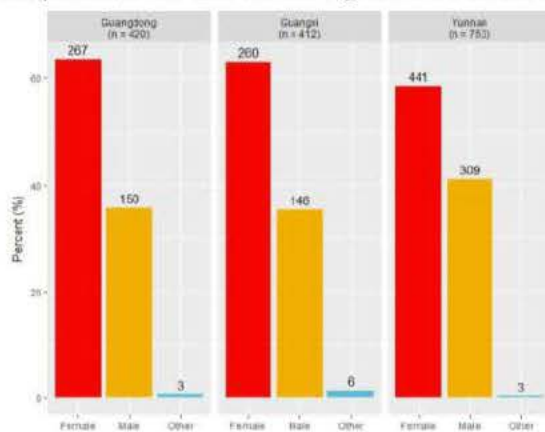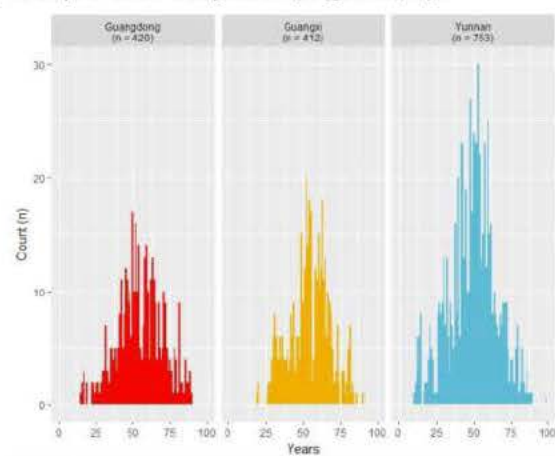


**Figure 1:** Gender of respondents



**Figure 2:** Age distribution of respondents.

Across all provinces, most respondents had lived in their respective locales for more than 5 years (96.3%) (**Fig. 3**) and earned less than 10,000 renminbi (RMB) annually (84.6%) (**Fig. 4**). In 2016, the updated poverty standard in China was 3,000 RMB as defined by Poverty Alleviation Office of State Council. More families in Guangxi (61.8%) lived at or below the poverty level as compared to those in Guangdong (36.9%) and Yunnan (43.3%).
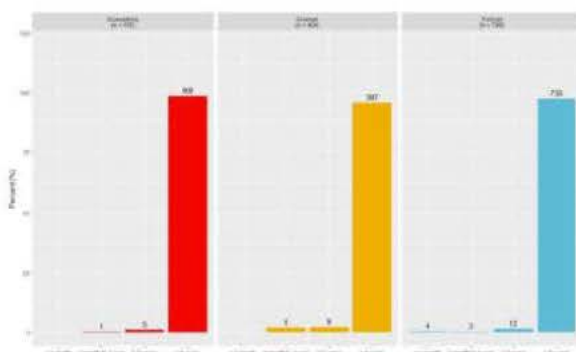


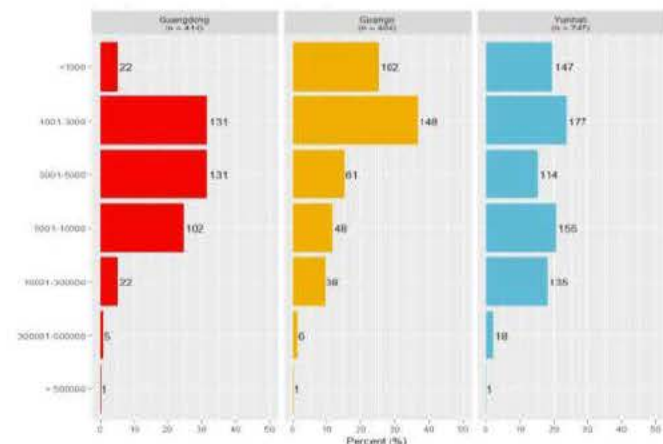**Figure 3:** Duration of residency.



**Figure 4:** Family annual per capita income (RMB).

2

1R01AI110964 Year 4 Report                                                PI: Daszak, Peter

In Guangdong, Guangxi, and Yunnan, 73.9%, 57.0% and 69.6% of respondents, respectively, had a primary school-level education or less **(Fig. 5)**. Across all provinces the most common livelihood was crop production. In Yunnan, 699 out of 753 (92.8%) individuals from the province identified crop production as a livelihood activity. In comparison, 237 out of 420 (56.4%) individuals from Guangdong, and 260 out of 412 (63.1%) individuals from Guangxi **(Fig. 6)** named crop production as a livelihood in the last year. Respondents, however, where not restricted to defining a single livelihood, many indicated engaging in multiple types of livelihoods.
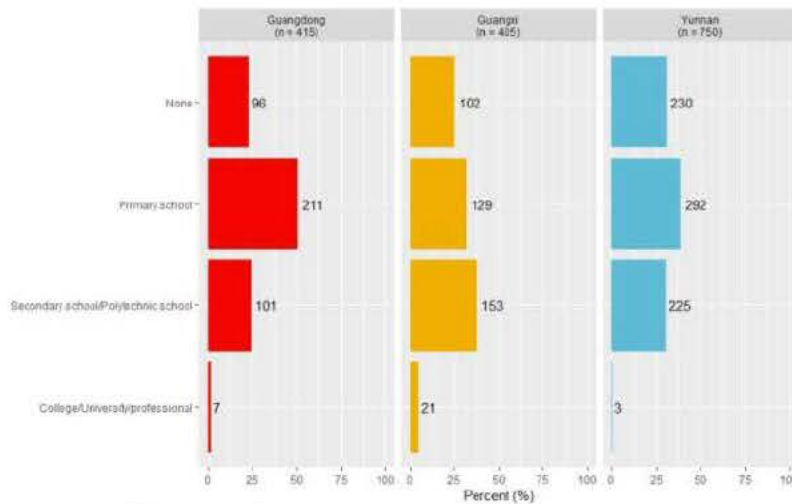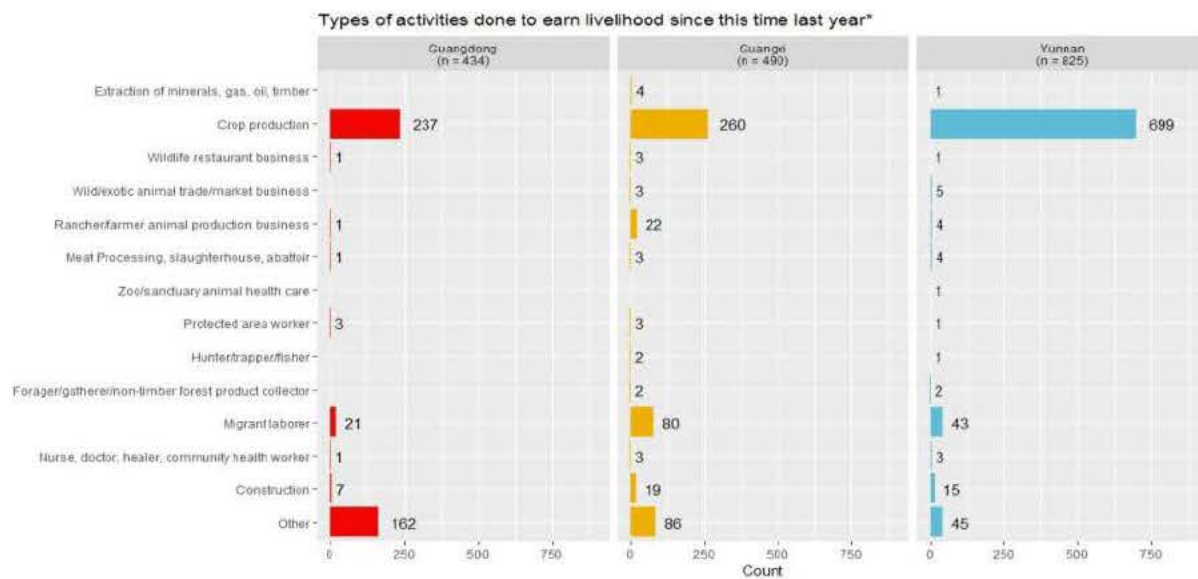


**Figure 5:** *Highest level of education completed*



**Figure 6:** *Types of activities conducted to earn a livelihood since this time last year (above)*

3

In Guangdong, Guangxi, and Yunnan, 41.7%, 50.7% and 59.6% of respondents, respectively, indicated that they traveled outside of their village town or city in the past year. Among those who traveled, the average number of trips was 5 in Guangdong and Guangxi, and 6 in Yunnan. The average distance traveled by respondents in Guangdong and Yunnan were 113 Km and 118 Km, respectively, compared to 66 Km by respondents in Guangxi.

## *Health-Seeking Behavior and Experiences with Unusual Illnesses*

When asked where they usually get treatment for illness or infection, the top 3 responses across all provinces in aggregate were hospitals, clinics, and pharmacies/dispensaries in descending order (Fig. 7). However, within Yunnan, most respondents went to hospitals, followed by pharmacies, then clinics.
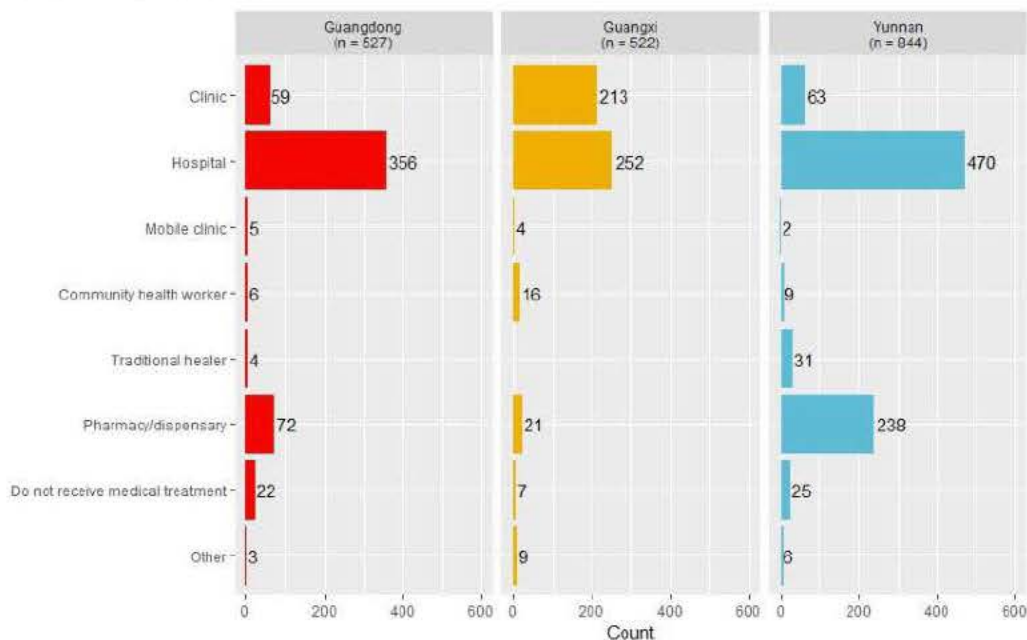


**Figure 7:** *Location where care was usually received for illness or infection.*

All survey respondents were asked whether they had experienced an unusual illness in their lifetime and in the past year, defined by a series of the most common symptoms associated with encephalitis, hemorrhagic fever (HF), severe acute respiratory infection (SARI), and influenza-like illness (ILI). Additional symptoms that were asked about included: fever with diarrhea or vomiting; fever with rash; and, persistent rash or sores on skin. Respondents were not restricted to selecting one illness and could provide multiple responses.

The proportion of respondents who had an unusual illness with any of the above-mentioned symptoms in their lifetime varied slightly by province. Between the three provinces, Yunnan had the fewest number of respondents who reported experiencing the symptoms provided (38.8%), compared to Guangdong and Guangxi (51.9% and 51.3%, respectively). Yunnan was also the only province where less than half of the respondents reported experiencing the symptoms provided (Fig. 8).

4

**Figure 8:** *Respondent's experience of unusual illnesses.*
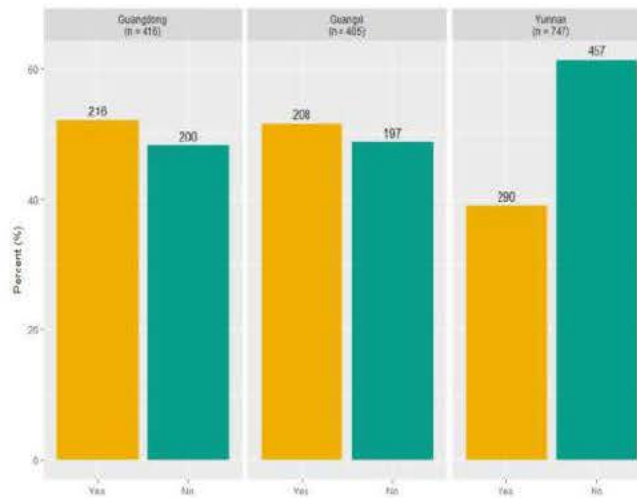


Across all three provinces, among those who had experienced any symptoms of unusual illness in their lifetimes, those associated with ILI were the most commonly reported. In Guangdong province, this was followed by symptoms associated with SARI, then by other symptoms not mentioned in the survey. In Guangxi province, the second most reported symptoms were ones associated with encephalitis, followed by other symptoms not mentioned in the survey. Similarly, in Yunnan, symptoms associated with encephalitis were the second most commonly reported, but this was followed by symptoms associated with SARI **(Fig. 9)**.

**Figure 9:** *Symptoms reported by people who had experienced unusual illness in their lifetime.*
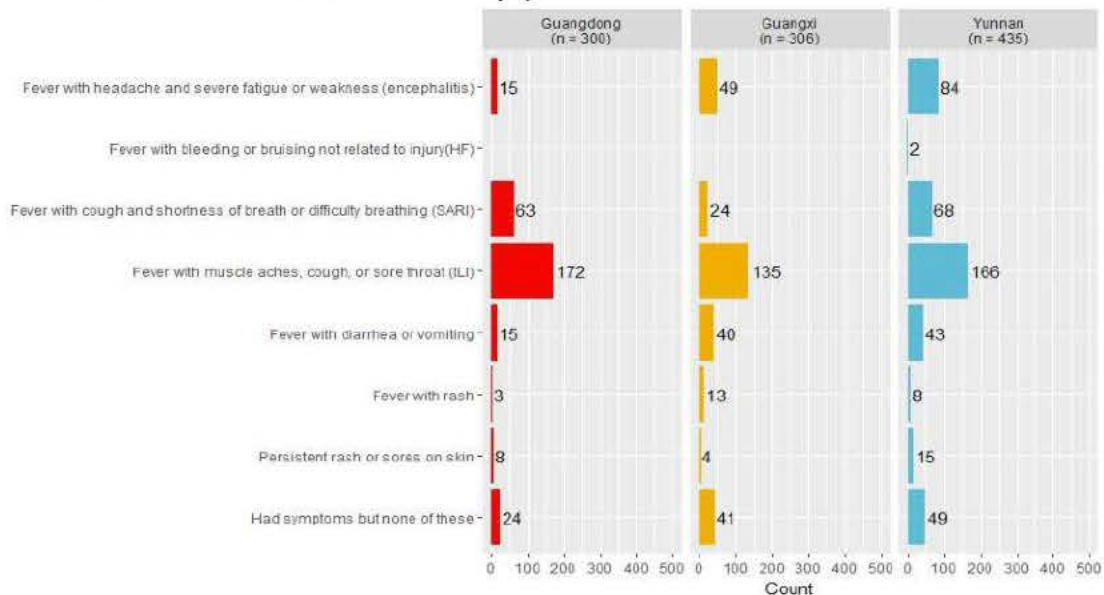


In each province, just under one-third of respondents who experienced the symptoms associated with an unusual illness in their lifetime indicated experiencing any of the symptoms in the past year – 22.2% in Guangdong, 32.8% in Guangxi and 23.0% in Yunnan **(Fig. 10)**.

5

**Figure 10:** *Whether respondents had experienced symptoms associated with an unusual illness, in the past year.*
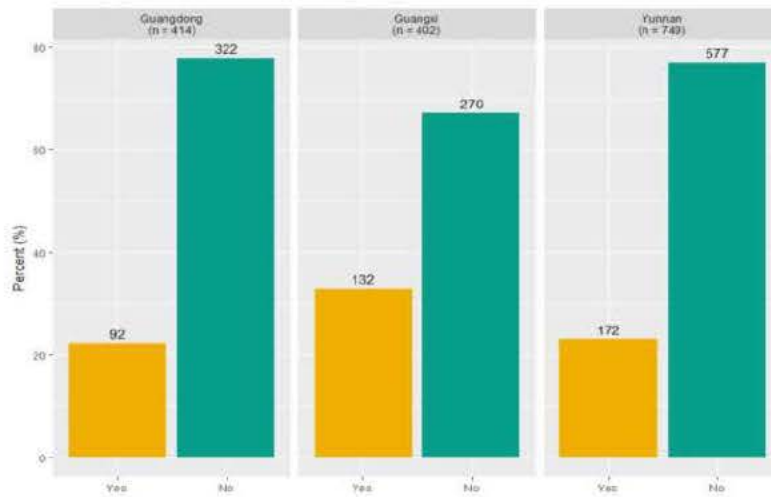


Of the respondents who reported having symptoms of unusual illness in the past year, across all three provinces, symptoms associated ILI were the most commonly reported. In Guangdong province, this was followed by symptoms associated with SARI then by other symptoms not provided in the survey. In Guangxi, symptoms associated with ILI were followed by symptoms associated with encephalitis, then by fever with diarrhea or vomiting. In Yunnan, symptoms associated with ILI were followed by symptoms associated with encephalitis, then by both SARI and other symptoms not provided in survey **(Fig. 11).**

**Figure 11:** *Symptoms experienced by those reporting unusual illness in the past year.*
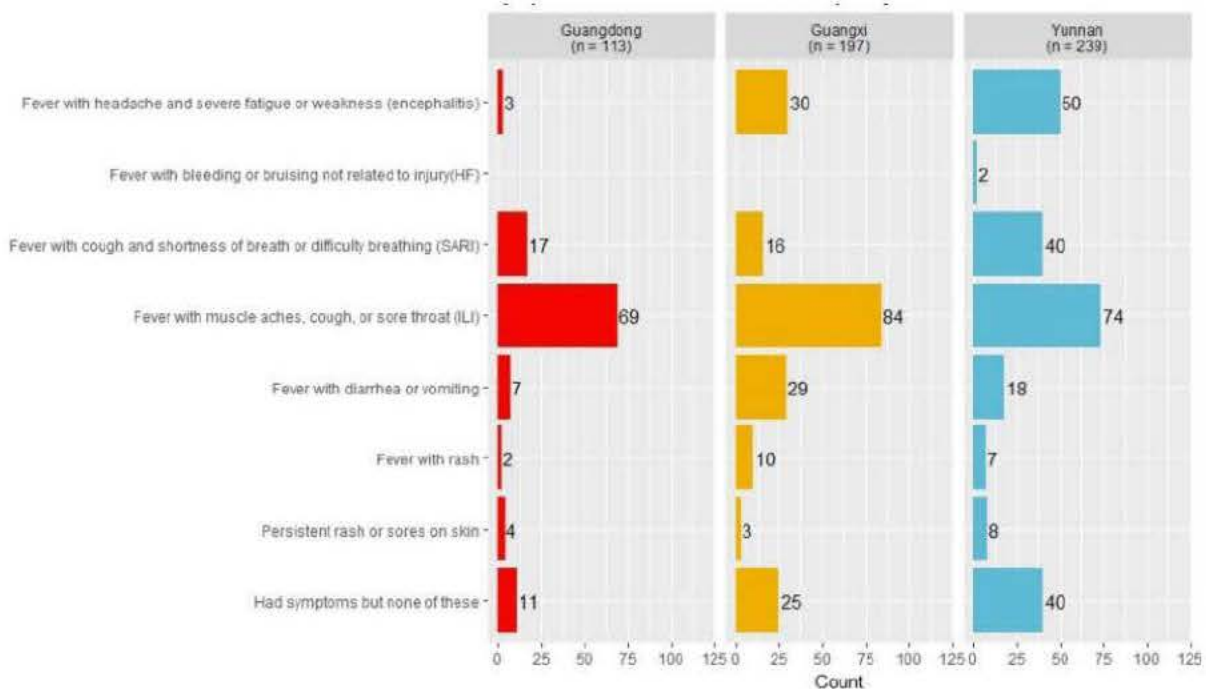


When respondents were asked what caused the symptoms associated with unusual illness experienced in the past year, 64.4% in Guangxi (85 of 132 respondents), and 50.0% in both Guangdong and Yunnan (46 of 92 respondents and 86 of 172, respectively), said they did not know the cause **(Fig. 12).** Only one respondent in Guangxi said their symptoms were due to

6

contact with animals (wild animals, specifically). Two respondents in Guangdong and one respondent in Guangxi said their symptoms were due to contact with animals (non-wild animals, specifically), whereas none of the respondents in Yunnan attributed their cause to contact with animals.
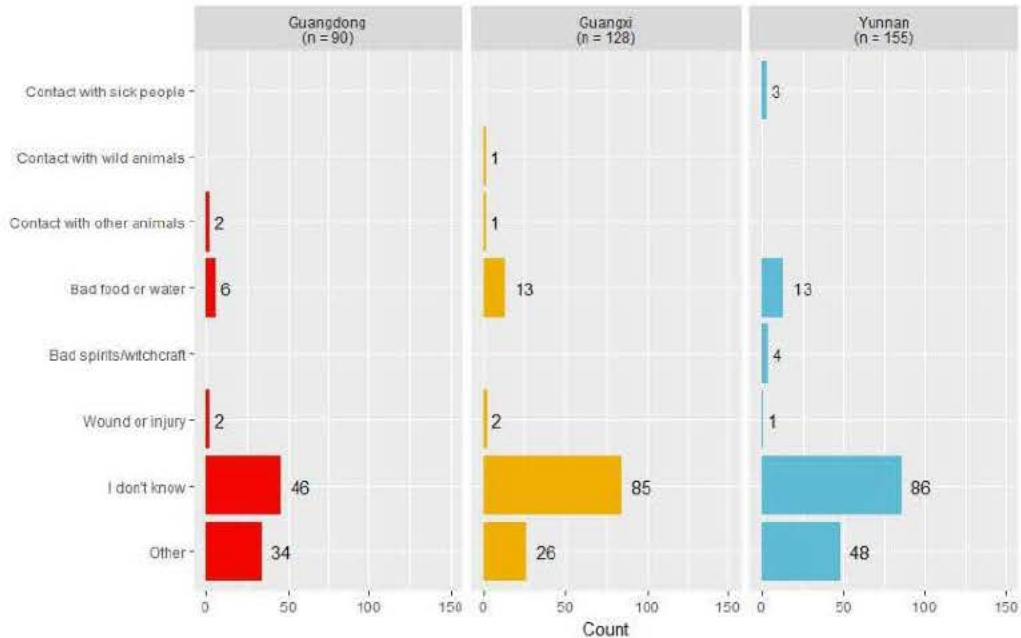


**Figure 12:** *Reported cause of sickness in the past year.*

Respondents reporting an unusual illness in the past year were asked if any of the people they lived with in the past year had symptoms similar to theirs, to assess possibilities of transmission among household members. Most respondents did not, across all three provinces: 82.4% in Guangdong, 83.6% in Guangxi and 79.9% in Yunnan (**Fig. 13**).

**Figure 13:** *Whether household members had similar symptoms of unusual illness, in the past year*



7
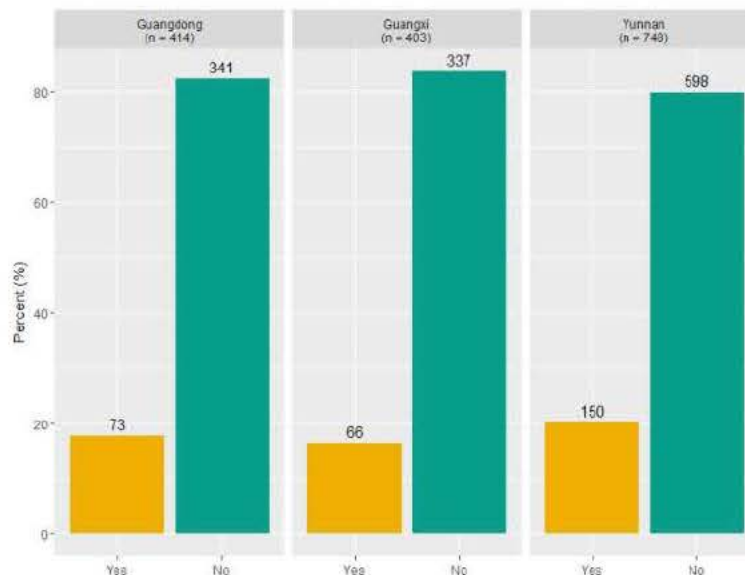
Of the household members who experienced symptoms of unusual illness in the past year, the most commonly reported symptoms were those associated with ILI **(Fig. 14)**.



**Figure 14:** *Symptoms of household members who were ill, in past year.*

Respondents were also asked if any members of their household who experienced symptoms of unusual illness died as a result of their illness in the past year. Across all the three provinces, almost none had died from these illnesses **(Fig. 15)**.



**Figure 15:** *Whether household members died from illness, in the past year.*
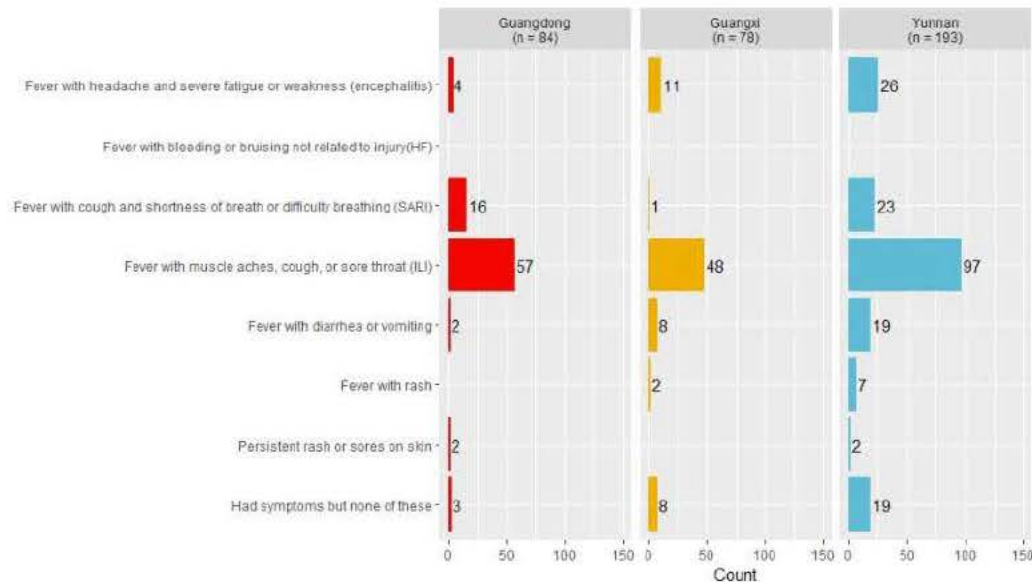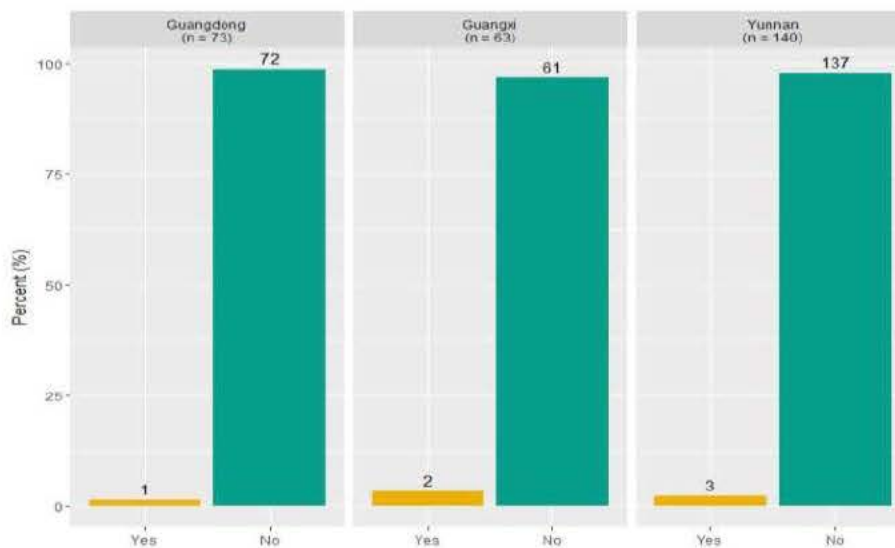
8

1R01AI110964 Year 4 Report                                             PI: Daszak, Peter

## *Contact with Animals*

All respondents were asked about various types of animal contacts in their lifetime and in the past year. More than two-thirds of the respondents across all provinces, as well as in each of the provinces, reported raising an animal within their lifetime (71.2% in Guangdong, 77.7% in Guangxi, and 97.7% in Yunnan). More than half of the respondents in each province reported having animals come inside their dwellings (83.1 % in Guangdong, 60.2% in Guangxi, and 92.5% in Yunnan). More than half of respondents in each province reported handling live animals (51.5 % in Guangdong, 56.9% in Guangxi, and 62.9% in Yunnan) **(Table 1)**. Respondents from Yunnan had more types of contact with animals in their lifetime than those from Guangdong and Guangxi. With the exception of cooking or handling meat, organs, or blood from a recently killed animal and being scratched or bitten by an animal, the proportion of respondents from Yunnan who engaged in all types of animal activities was higher than the other provinces.

| Type of animal contact (past year) | Guangdong | | Guangxi | | Yunnan | |
|---|---|---|---|---|---|---|
| | (n) | (%) | (n) | (%) | (n) | (%) |
| Lived with an animal as a pet | 43 | 100 % | 72 | 98.6 % | 335 | 100 % |
| Handled live animals | 212 | 100 % | 226 | 98.3 % | 332 | 99.7 % |
| Raised a live animal | 296 | 100 % | 312 | 99.4 % | 518 | 99.8 % |
| Shared water source with animals for washing | 47 | 100 % | 19 | 95.0 % | 97 | 100 % |
| Seen animal feces in or near food before you have eaten it | 18 | 100 % | 15 | 93.8 % | 43 | 100 % |
| Eaten food after an animal has touched or damaged it | 6 | 100 % | 6 | 100 % | 29 | 100 % |
| Animals come inside the dwelling where you live | 345 | 100 % | 239 | 98.0 % | 493 | 100 % |
| Cooked or handled meat, organs, or blood from a recently killed animal | 333 | 100 % | 144 | 97.3 % | 412 | 100 % |
| Eaten raw or undercooked meat or organs or blood | 2 | 100 % | 25 | 89.3 % | 65 | 98.5 % |
| Eaten an animal that was not well/sick | -- | -- | 1 | 100 % | 6 | 100 % |
| Found a dead animal and collected it to eat, share, or sell | -- | -- | 3 | 100 % | 10 | 100 % |
| Been scratched or bitten by an animal | 1 | 100 % | 31 | 100 % | 28 | 96.6 % |
| Slaughtered an animal | 145 | 100 % | 69 | 98.6 % | 303 | 100 % |
| Hunted or trapped an animal | 9 | 100 % | 4 | 100 % | 22 | 95.7% |

**Table 1:** *Types of animal contact, <u>within a respondent's lifetime</u>.*

Respondents who reported having animal contact in their lifetime were also asked to indicate if they had the same type of animal contact <u>in the past year</u> **(Table 2)**. In the past year, across all three provinces and in each province, almost all respondents engaged in all contact types with the exception of eating an animal that was not well/sick, and finding a dead animal and collecting it to eat, share, or sell (0% for both in Guangdong).

9

| Type of animal contact (lifetime) | Guangdong | | Guangxi | | Yunnan | |
|---|---|---|---|---|---|---|
| | (n) | (%) | (n) | (%) | (n) | (%) |
| Lived with an animal as a pet | 43 | 10.4 % | 73 | 18.1 % | 335 | 62.9 % |
| Handled live animals | 212 | 51.5 % | 230 | 56.9 % | 334 | 62.8 % |
| Raised a live animal | 296 | 71.2 % | 314 | 77.7 % | 521 | 97.7 % |
| Shared water source with animals for washing | 47 | 11.5 % | 21 | 5.2 % | 97 | 18.2 % |
| Seen animal feces in or near food before you have eaten it | 18 | 4.4 % | 16 | 3.9 % | 43 | 8.1 % |
| Eaten food after an animal has touched or damaged it | 6 | 1.5 % | 6 | 1.5 % | 29.0 | 5.4 % |
| Animals come inside the dwelling where you live | 345 | 83.1 % | 244 | 60.2 % | 493 | 92.5 % |
| Cooked or handled meat, organs, or blood from a recently killed animal | 333 | 80.4 % | 148 | 36.7 % | 413 | 77.5 % |
| Eaten raw or undercooked meat or organs or blood | 2 | 0.5 % | 28 | 6.9 % | 68 | 12.8 % |
| Eaten an animal that was not well/sick | -- | -- | 1 | 0.3 % | 6 | 1.1 % |
| Found a dead animal and collected it to eat, share, or sell | -- | -- | 3 | 0.7 % | 10 | 1.9 % |

**Table 2:** *Types of animal contact, in past year.*

Respondents who had animal contact in the past year were asked to identify the animals involved in the interaction. *(Figs. 16-26, below: the first two figures are enlarged to show row labels, which are identical for all)*. Cats and dogs were the most common pets reported across all provinces and in each province (**Fig. 16b**).
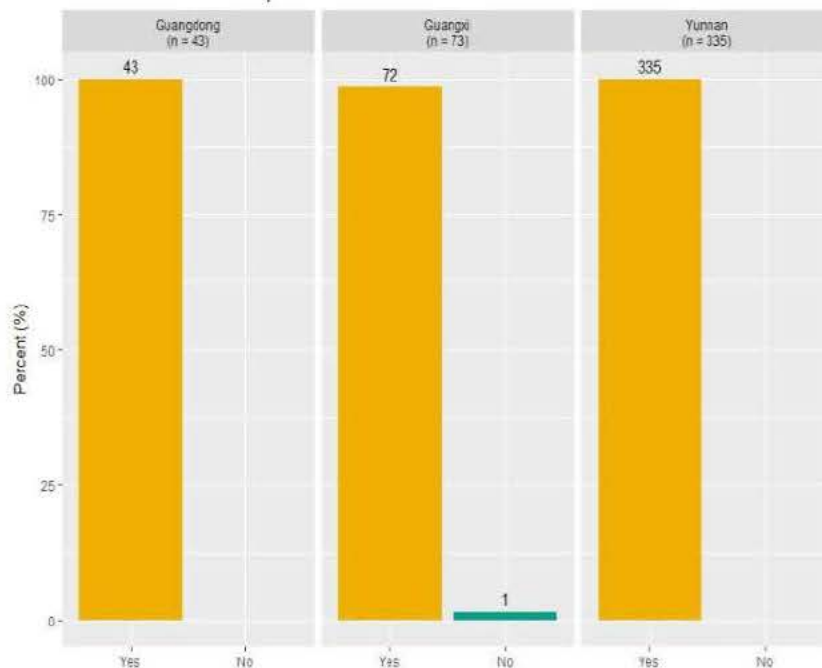


**Figure 16a (top) & b (below):** *(a) Whether respondents had lived with an animal as a pet, in the past year, and (b) among those who had, types of animal kept as pets.*
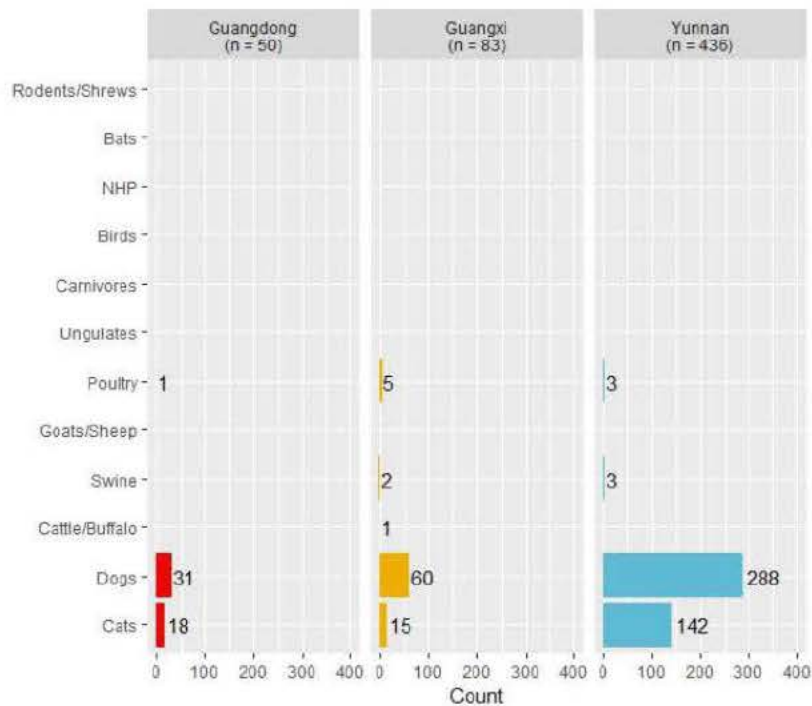
Poultry was the most common type of animal handled across all provinces as well as in each province, with 96.2%, 90.3%, and 92.8% of respondents handling animals in Guangdong, Guangxi and Yunnan, respectively (Fig. 17b).



**Figure 17a & b:** (a) Whether respondents had handled live animals, in the past year, and (b) among those who had, types of live animals handled.

11

Poultry was also the most commonly raised animal in each of the three provinces; 95.3%, 87.5%, 95.4% in Guangdong, Guangxi, and Yunnan, respectively **(Fig. 18b)**.



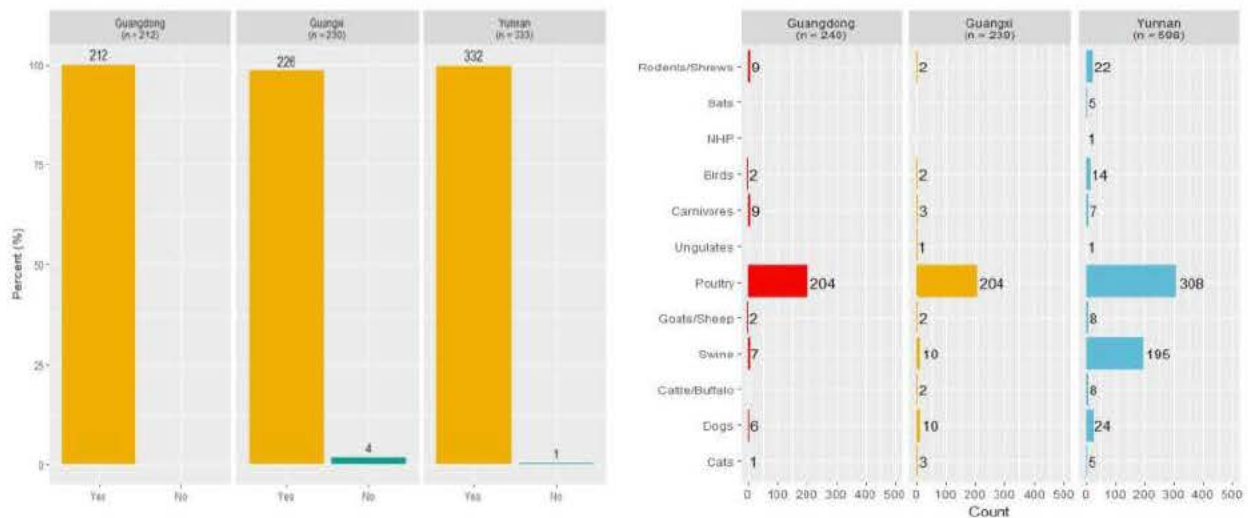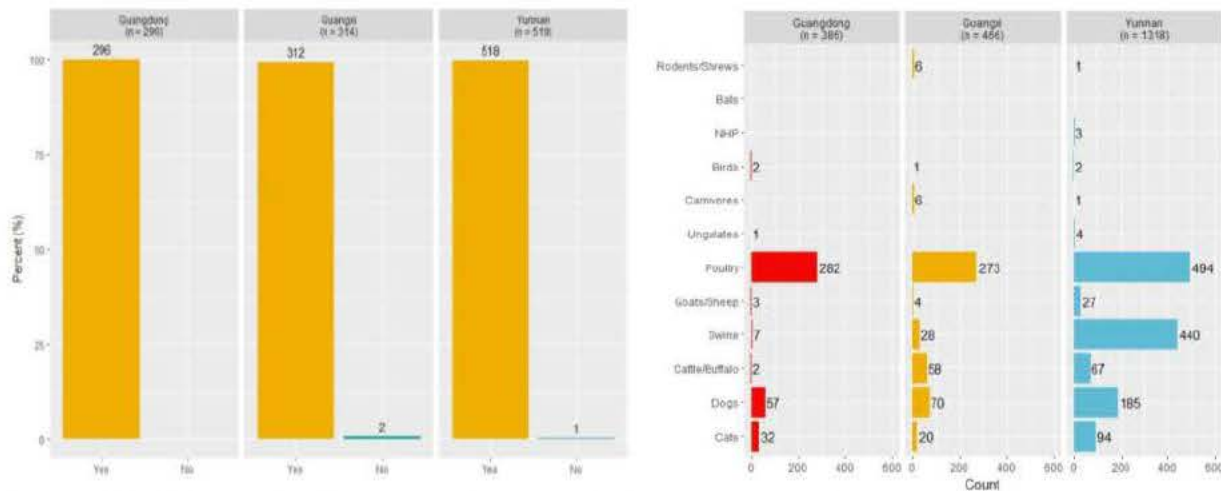**Figures 18a & b:** *(a) Whether respondents had raised live animals in the past year, and (b) among those who had, types of animals raised.*

In all three of the provinces, the most common type of animals found in respondent dwellings were rodents or shrews. In Guangdong and Yunnan, birds were the second most common animal type found in dwellings. In Guangxi province, birds along with poultry were the second most common animal type. Respondents in Guangdong and Yunnan reported that all 12 animal taxa had come inside their dwellings in the past year. Taxa seen in the dwellings of respondents from Guangdong and Yunnan and not Guangxi were non-human primates, ungulates, goats or sheep, swine, and cattle or buffalo **(Fig. 20b)**.
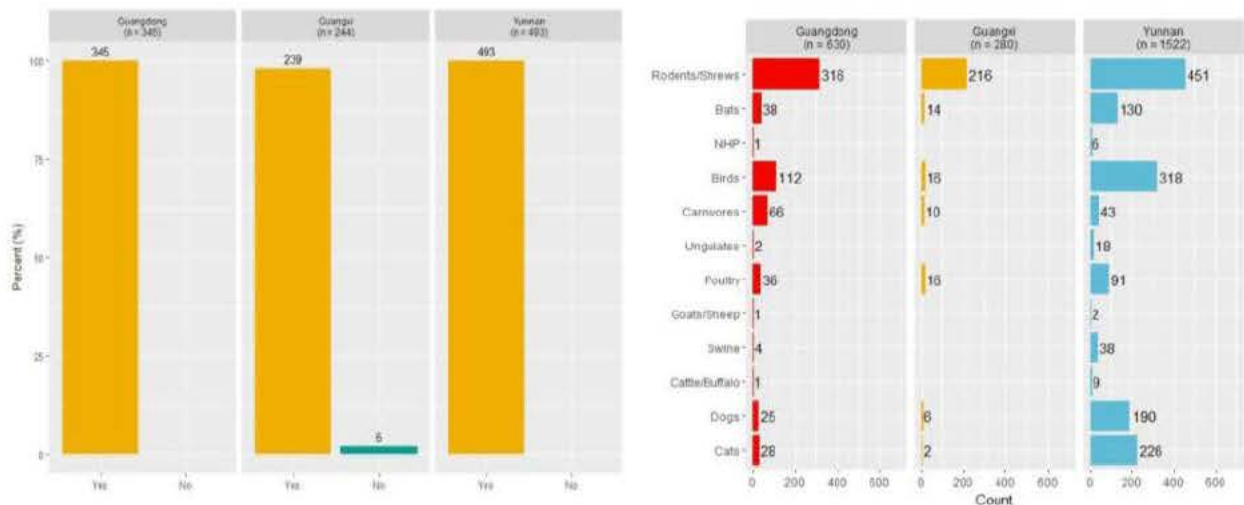


**Figure 19a & b:** *(a) Whether respondents had animals come inside dwelling, in the past year, and (b) among those who had, types of animals in dwelling.*

12

Almost all of the respondents who said they have cooked or handled meat, organs, or blood in their lifetime reported doing so in the past year. Common animal types that were cooked handled included poultry and swine in all three provinces (**Fig. 20**).
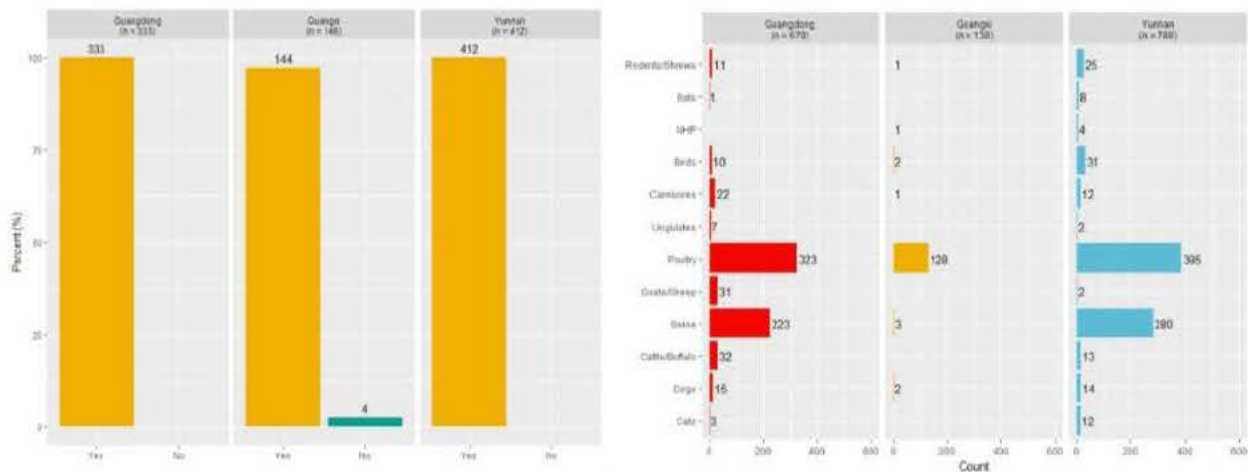


***Figure 20a & b***: *(a) Whether respondents had cooked or handled meat, organs or blood from a recently killed animal, in the past year, and (b) among those who had, types of animals whose meat, organs or blood was cooked or handled.*

More respondents in Yunnan reported eating raw or undercooked meat compared to respondents in Guangdong and Guangxi (**Fig. 21**). In Yunnan, 96% of respondents who ate raw or undercooked meat in their lifetime did so in the past year. The types of animal products that were eaten raw or undercooked by respondents in Yunnan were mostly from swine. In Guangxi, the most commonly reported type of animal meat that had been eaten raw or undercooked was that of carnivores.



***Figure 21 a & b***: *(a) Whether respondents had eaten raw or undercooked meat or organs or blood, in the past year, and (b) among those who had, types of animals whose meat, organs or blood were eaten raw or undercooked.*

13

Across all provinces, a total of 13 respondents in Guangxi and Yunnan indicated that they collected an animal that was found dead to eat, share or sell. In Guandong, no respondents reported finding a dead animal and collecting it to eat, share, or sell. The most common type of animal collected across all provinces in aggregate was poultry. In Yunnan, poultry was the most common type of animal found dead and collected to eat, share or sell (80.0%), whereas dogs were the most common type in Guangxi (66.7%) (**Fig. 22**).



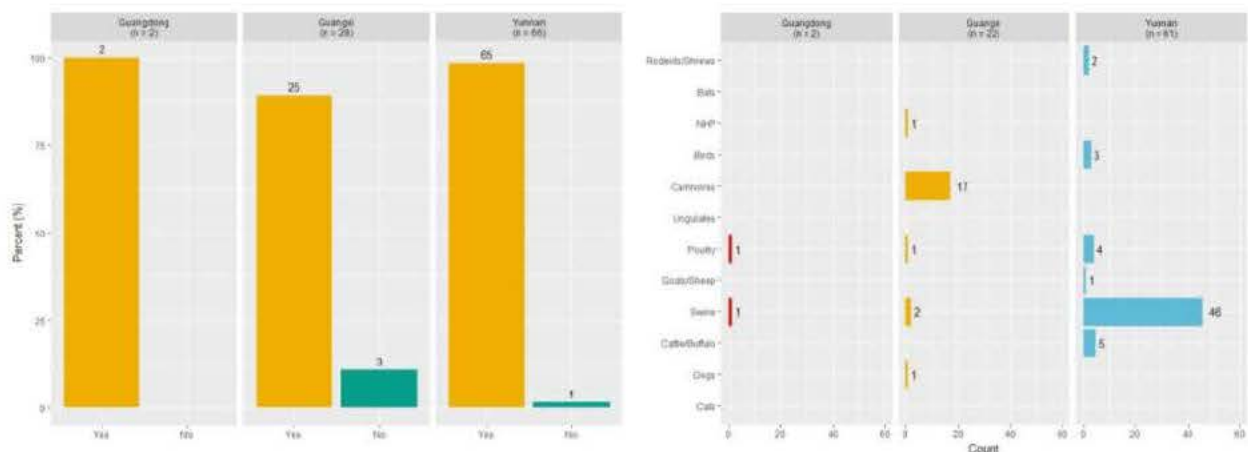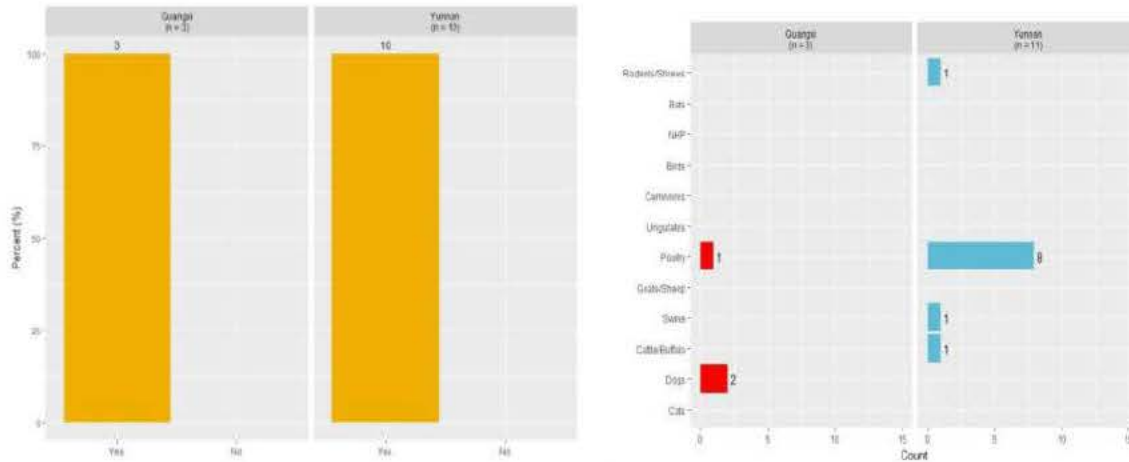**Figure 22 a & b:** (a) Whether respondents had found a dead animal and collected it to eat, share, or sell, in the past year, and (b) among those who had, types of animals that were found dead and collected to eat, share, or sell.

In each province, almost all of the respondents who indicated being scratched or bitten by an animal in their lifetime said it occurred in the past year (100% in Guangdong, 98.6% in Guangxi, and 100% in Yunnan). In both Guangxi and Yunnan, dogs were the common type of animal that respondents said they were scratched or bitten by (64.5% in Guangxi and 50.0% in Yunnan). Cats were the second most common in Guangxi and Yunnan (9.6% in Guangxi, and 28.5% in Yunnan). Across all three provinces, only one respondent from Yunnan said that they were scratched or bitten by a bat (**Fig. 23**).



**Figure 23 a & b:** (a) Whether respondents had been scratched or bitten by an animal, in the past year, and (b) among those who had, types of animals that scratched or bit respondents.

14

PI: Daszak, Peter

Poultry was the most common type of animal slaughtered during the past year across all provinces as well as in each province (95.8% in Guangdong, 79.7% in Guangxi, and 94.1% in Yunnan). In addition to poultry, respondents in Yunnan also commonly only slaughtered swine (43.9%), compared to 1.4% in Guangdong and 7.3% in Guangxi **(Fig. 24)**.
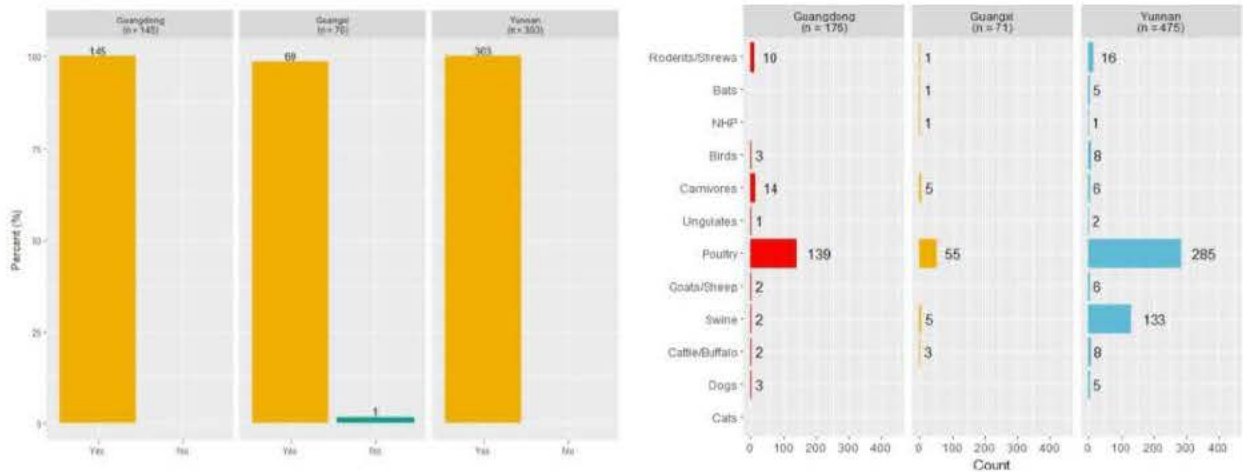


**Figure 24 a & b:** (a) Whether respondents had slaughtered an animal, in the past year, and (b) among those who had, types of animals slaughtered.

Carnivores were the most common taxa of animals hunted or trapped in the past year, in Guangdong and Guangxi. In Yunnan, rodents or shrews and birds were reported as the most common. Bats, non-human primates and dogs were animal types hunted by respondents in Yunnan but not by respondents in Guangdong and Guangxi **(Fig. 25)**.



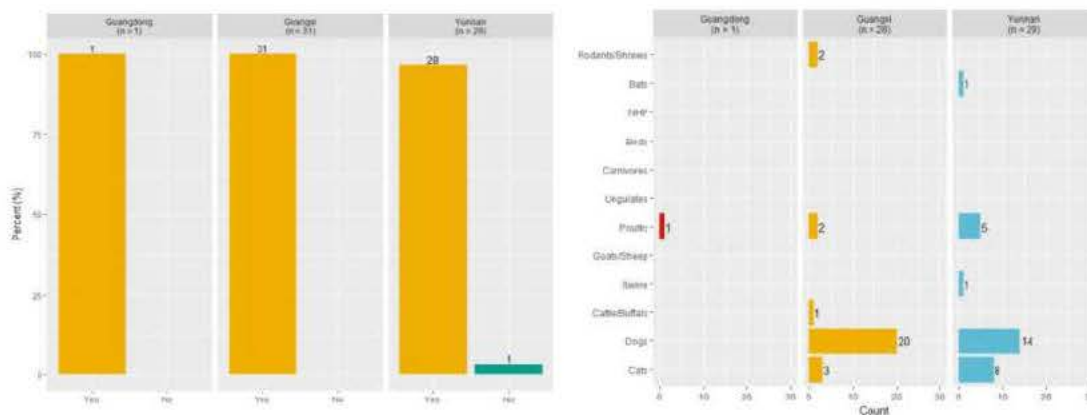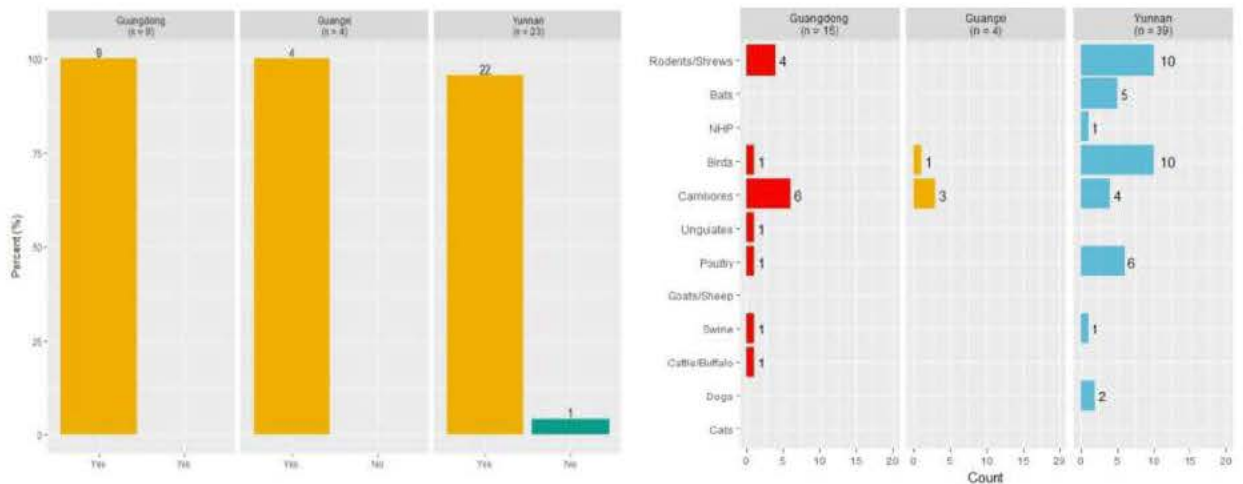**Figure 25 a & b:** (a) Whether respondents had hunted or trapped an animal, in the past year, and (b) among those who had, types of animals hunted or trapped.

15

In examining bat-specific contact, across all provinces and within each province, the most common interaction with bats was finding them inside their houses. Respondents in Yunnan also hunted/trapped and handled bats, and were scratched/bitten by bats, whereas these did not occur in Guangdong or Guangxi **(Fig. 26)**.
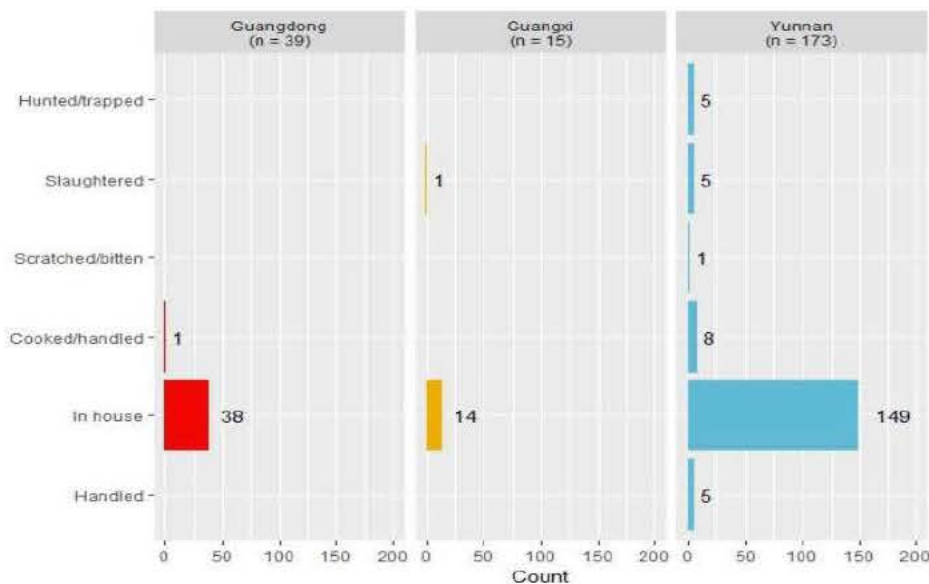


**Figure 26:** *Types of bat contact.*

After respondents were asked about their contact with wildlife and livestock, they were asked about their knowledge of whether animals can spread diseases and whether they were worried about diseases and disease outbreaks at wet markets. The proportion of respondents who thought that animals can spread disease was highest in Guangdong province (72.3%). In Guangxi and Yunnan, the proportion of those who thought animals could spread disease compared to those who thought that they did not were roughly equivalent – 47.5% versus 50.7% in Guangxi and 49.2% versus 49.3% in Yunnan **(Fig. 27)**.
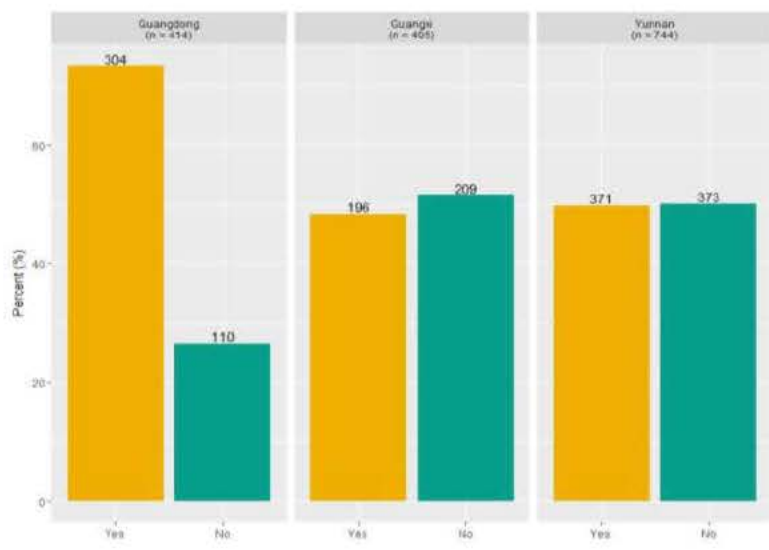


**Figure 27:** *Whether respondents thought that animals can spread disease.*

16

Similarly, when respondents were asked about whether they were worried about diseases or disease outbreaks in animals at wet markets, Guangdong had the highest proportion of respondents who said they were worried (67.3%). In both Guangxi and Yunnan, the proportion of respondents that was not worried (57.5% and 51.5%, respectively) was higher than the proportion that was worried **(Fig. 28)**
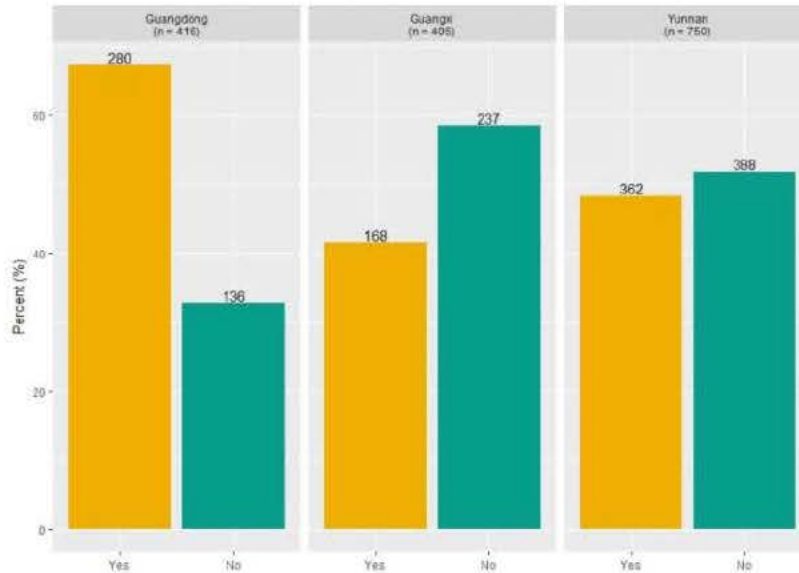


*Figure 28:* Whether respondents were worried about diseases or disease outbreaks in animals at wet markets.

## Serological Evidence of Bat SARS-related CoV Infection in Humans

Respondents were asked to provide a biological sample to assess whether SARS-CoV spillover had occurred at the high-risk location where the survey has been implemented. A total of 1,530 serum samples were collected from 2016 to 2017 from individual residents in villages close to bat caves where coronaviruses were previously detected.

We developed an ELISA serology test using the purified NP protein of MERS-CoV, SARSr-CoV, HKU9 CoV and HKU10 CoV as coating antigen respectively and using Anti-Human IgG Monoclonal antibody as secondary antibody. All sera were screened for antibodies against these 4 bat-origin coronaviruses. Anti-SARSr-CoV NP IgG was detected in 10 samples, and 6 samples were positive for IgG against HKU10 NP. The 16 ELISA positive samples were further tested by confirmatory western blot, 7 samples from Yunnan province were confirmed positive for anti-SARSr-CoV, two samples (one from Guangdong province and one Guangxi province) were confirmed positive for anti-HKU10 **(Table 3)**.

17

| Locations | | Sample No. | NP Antibody Positive No. | | | |
|---|---|---|---|---|---|---|
| | | | HKU9 CoV | MERS CoV | SARSr-CoV | HKU10 CoV |
| Yunnan (2016) | Jinning | 209 | | | *6 | |
| | Mengla | 168 | | | 2 (*1) | |
| | Jinghong | 212 | | | | 2 |
| | Lufeng | 144 | | | | |
| Guangdong (2016) | Zengcheng | 234 | | | 1 | 2 |
| | Ruyuan | 179 | | | | |
| Guangxi (2017) | Mashan | 160 | | | 1 | |
| | Guilin | 224 | | | | *2 |
| Total | | 1,530 | 0 | 0 | *7 | *2 |

**Table 3** *Results of ELISA testing of human sera for antibodies to 4 different bat CoV species (*confirmed with western blot).*

<u>Links Between ELISA Results and Behavior</u>
Only one out of the seven SARS-related CoV seropositive respondents said that they had an unusual illness in their lifetime with reported symptoms similar to encephalitis or neural involvement. Two of the respondents said they had experienced symptoms in the past year with only one respondent specifying that they experienced epigastric pain and dizziness. The seven seropositive SARSr-CoV respondents reported various types of animal contacts in the past year. Three had lived with an animal as a pet, four handled a live animal, four raised a live animal, five saw animals inside their dwellings, five had cooked or handled meat, organs, or blood from a recently killed animals, one ate an animal that they knew was not well or sick, one was scratched or bitten by an animal, and four had slaughtered an animal. The only bat contact reported was by one respondent who saw a bat in their dwelling.

Both of the respondents who tested positive for HKU10-CoV antibodies said they had experienced an unusual illness in their lifetime, with symptoms associated with encephalitis and SARI. Neither respondent had experienced any symptoms of unusual illness in the past year. Both had reported handling and raising animals, with one indicating they saw animals come inside their dwelling, and one indicating cooking or handling meat, organs, or blood from a recently killed animal. No bat contact was reported by either of the respondents. Overall, five of the total nine SARS-related CoV and HKU10-CoV seropositive respondents reported being worried about disease or disease outbreaks at wet markets. Seven of the nine reported purchasing live animals from a wet market.

**Specific Aim 1: Summary of Key Findings**
Our analysis of the key risk factors relating to potential viral zoonotic disease spillover in China indicated some notable differences among the respondents in Guangdong, Guangxi, and Yunnan.  With respect to demographic factors, Guangxi fared the lowest on key socio-economic

18

status indicators when compared to Guangdong and Yunnan provinces as reflected by the higher proportion of respondents in Guangxi living under the poverty level.

When assessing the type of animal contact and the associated animal taxa over the course of a respondent's lifetime, the results show that respondents in Yunnan engaged in greater contact with animals then those from Guangdong and Guangxi. For example, for 12 of the 14 animal contact types, a higher proportion of Yunnan respondents engaged in these respective activities than in Guangdong and Guangxi. Respondents in Yunnan also reported hunting bats, dogs, and non-human primates which were not reported to being hunted in Guangdong and Guangxi. Swine contact was higher in Yunnan for handling, raising, and slaughtering activities. When examining the various types of animal contact associated with bats only, our results also show that Yunnan respondents reported more varied types of contact with bats. Respondents in Yunnan indicated handling, being scratched by, slaughtering, and hunting bats, but these interactions did not occur in Guangdong or Guangxi. Additional analyses that examine predictors of animal contact in each province will be the focus of human behavioral analyses in Year 5 of the study.

Even though our sample population lives in areas that have dense and diverse bat populations, our results show an overall low proportion of respondents reporting hunting and trapping bats in all three provinces. The low proportion of hunting practice could be attributed to the success of conservation enforcement efforts undertaken by the government. These efforts may have effectively reduced the illegal practice of hunting wildlife or, as a consequence, moved the activity underground which made respondents less forthcoming about revealing their engagement in such practices. Further investigation into the potential causes is also warranted.

Our analyses also reveal differences in perceptions associated with zoonotic disease spillover between Guangdong, and Guangxi and Yunnan. For example, the proportion of respondents who thought that animals can spread disease was highest in Guangdong province at 72.3%, as compared to Guangxi (48.3%) and Yunnan (49.9%). Moreover, about two-thirds of respondents in Guangdong were worried about diseases and disease outbreaks in wet markets. These differences in perception observed in Guangdong compared to Guangxi and Yunnan could potentially be attributable to a heightened awareness of zoonotic disease emergence due to the 2001 SARS outbreak.

Finally, our serological testing results provide the first evidence ever of a bat SARSr-CoV spilling over into people in the wild. All of the SARSr-CoV positive individuals were from Yunnan province, which is the site of a cave in which we have identified a large diversity of SARSr-CoVs within the virome of which every genetic element of SARS-CoV can be identified. These findings warrant further investigations into the type of exposures that may have contributed to bat SARS-related CoVs to infect humans in this particular region. **They also highlight this region as a hotspot for SARSr-CoV future spillover risk.**

## 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 rectal swab and oral swab samples from 671 individual bats from 20 species in Guangdong and Guangxi provinces in southern China in Year 4 **(Table 4)**. 671 rectal swab samples were tested for CoV RNA and 154 (23.0%) were positive **(Table 5)**.

| Date of Sampling | Sampling Locations | Rectal swabs | Oral swabs |
|---|---|---|---|
| May 10th 2017 | Hezhou, Guangxi | 6 | 6 |
| May 11-12th 2017 | Chongzuo, Guangxi | 67 | 67 |
| May 13th 2017 | Nanning, Guangxi | 66 | 66 |
| May 17th, 2017 | Beihai, Guangxi | 23 | 23 |
| May 19th 2017 | Chongzuo, Guangxi | 36 | 36 |
| May 21st 2017 | Yangshan, Qingyuan, Guangdong | 46 | 46 |
| May 22nd, June 7th 2017 | Huidong, Huizhou, Guangdong | 103 | 103 |
| June 9th 2017 | Nanning, Guangxi | 71 | 71 |
| June 9th 2017 | Ningming, Chongzuo, Guangxi | 63 | 63 |
| September 10th 2017 | Huidong, Huizhou, Guangdong | 100 | 100 |
| September 11th 2017 | Yingde, Guangdong | 90 | 90 |
| **Total** | | **671** | **671** |

**Table 4.** *Bat samples collected for CoV surveillance in Year 4*

| Species | Guangdong | Guangxi | Total |
|---|---|---|---|
| *Rhinolophus sinicus* | 9/27 | 6 | 9/33 |
| *Rhinolphus rex* | | 4 | 4 |
| *Rhinolophus pusilus* | 1 | 2 | 3 |
| *Rhinolophus pearsoni* | 5 | | 5 |
| *Hipposideros armiger* | 24 | 8 | 32 |
| *Hipposideros larvatus* | 9 | 9 | 18 |
| *Hipposideros pomona* | | 20 | 20 |
| *Hipposideros pratti* | 26 | | 26 |
| *Aselliscus stoliczkanus* | | 1 | 1 |
| *Miniopterus fuliginosus* | 1 | | 1 |
| *Miniopterus pusillus* | 29/39 | | 29/39 |
| *Myotis chinensis* | 2/27 | | 2/27 |
| *Myotis daubentonii* | 2 | | 2 |
| *Myotis ricketti* | 86/178 | | 86/178 |
| *Pipistrellus abramus* | | 2 | 2 |
| *Pipistrellus pipistrellus* | | 2 | 2 |
| *Scotophilus kuhli* | | 24/137 | 24/137 |
| *Tylonycteris pachypus* | | 4/115 | 4/115 |
| *Tylonycteris robustula* | | 3 | 3 |
| *Cynopterus sphinx* | | 23 | 23 |
| **Total** | **126/339** | **28/332** | **154/671** |

**Table 5**. *Number of bat specimens tested and positive (bold) in Year 4*

20

A high prevalence of HKU6-related coronaviruses (48.3%), Scotophilus coronavirus 512 (17.5%), and coronavirus 1B (71.8%) was detected in *Myotis ricketii*, *Schotophilus khulii* and *Miniopterus pusillus*, respectively. SARS-related coronaviruses and HKU2-related coronaviruses were discovered in 4 and 5 *Rhinolophus sinicus* samples respectively from Guangdong. HKU4 coronaviruses were identified in 4 *Tylonycteris pachypus* from Guangxi (**Fig. 29**).
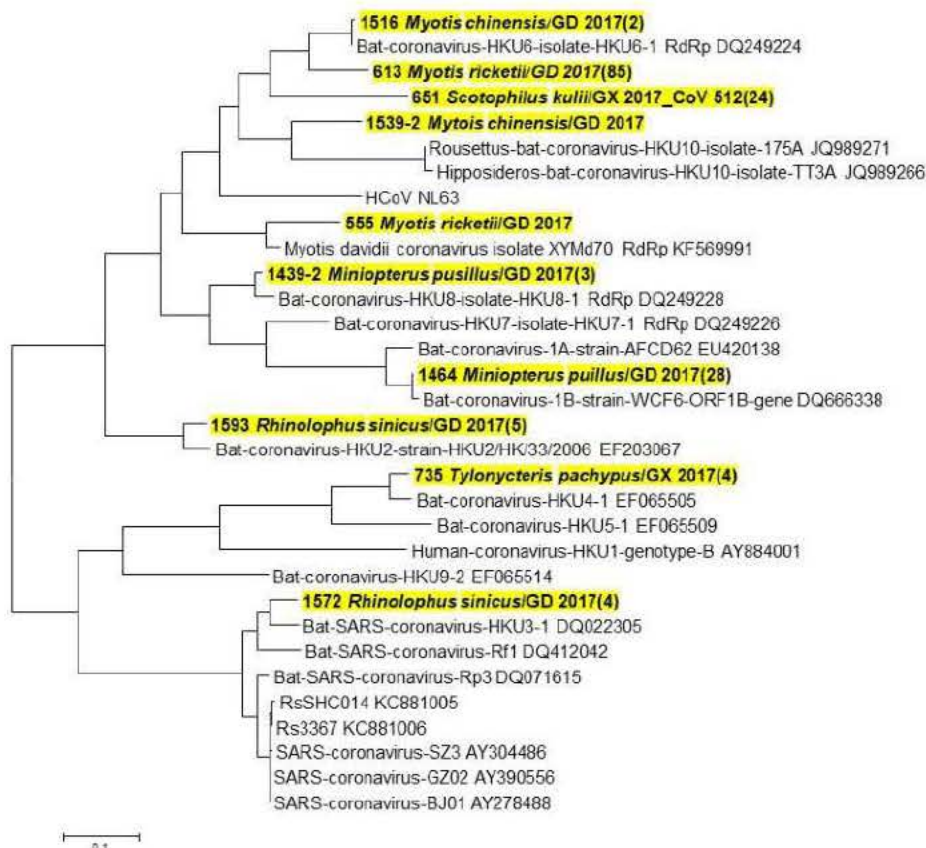


**Figure 29:** *Phylogenetic analysis of partial RdRp gene of CoV (440-nt partial sequence)*

**Genetic Diversity and Genomic Characterization of Betacoronaviruses in Fruit Bats**
In Year 4, we analyzed the genetic diversity of betacoronaviruses we have detected since 2009 in different species of fruit bats in Yunnan province, including *Eonycteris spelaea*, *Rousettus leschenaultia* and an unclassified *Rousettus* species. These viruses are classified into two betacoronavirus species, HKU9-CoV and GCCDC1-CoV. All HKU9-related viruses (n=46) were found in *Rousettus* spp. bats while GCCDC1-related viruses (n=13) from *E. spelaea*. Phylogenetic analysis of the full-length N gene suggests that HKU9-related CoVs are highly diverse and divided into 5 lineages with previously reported strains, and the GCCDC1-related CoVs were more similar between each other (**Fig. 30**).

The full-length genome sequence of a novel HKU9-related CoV termed 2202 was determined. It shares 83% nt identity with other HKU9 strains, with the most divergent regions located in the S

21

1R01AI110964 Year 4 Report                                         PI: Daszak, Peter

protein, but shares only 68% aa identity with those of other HKU9 strains. Virus quantification revealed that intestine was the primary infected organ for HKU9-related CoVs while kidney and lungs could also be target tissues, suggesting potential for spillover through oral-fecal, respiratory, or uro-genitary routes.
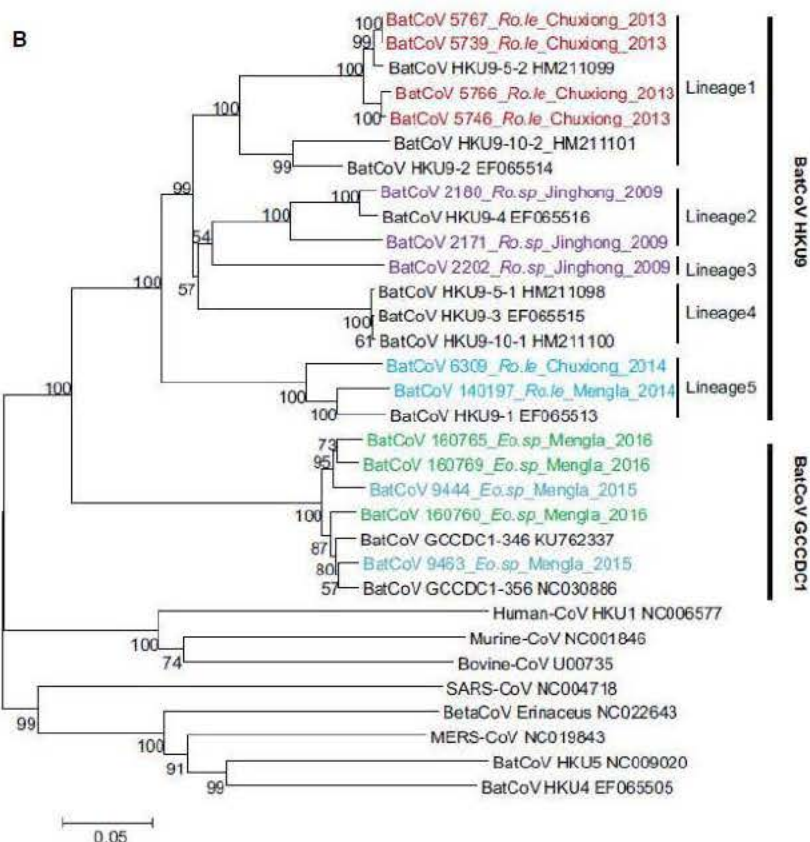


**Figure 30.** *Phylogenetic analysis of full-length N gene of HKU9 and GCCDC1 CoVs*

## Bat Coronavirus Host-Virus Phylogeography in China

We used discrete ancestral character state reconstruction to estimate viral history and reconstructed the inferred bat host genus for each node within the phylogenetic tree **(Figs. 31, 32)**. The color of tree branches indicates the inferred ancestral host bat genus for the reconstructed phylogeny. *Rhinolophus* is the inferred ancestral host of lineages B and C (SARS-like CoVs and MERS-like CoVs, respectively). This genus played an important role in the diversification of Beta-CoVs. A larger host diversity is observed for Alpha-CoVs. Our dataset for this analysis includes all CoV RdRp sequences isolated from bat specimens collected by our team from 2008-2015 (Alpha-CoVs: n = 491 – Beta-CoVs: n = 326), including those collected under prior NIAID funding (1 R01 AI079231), funding from Chinese Federal Agencies, and a large majority from our current NIAID project. All Chinese bat CoV RdRp sequences available in GenBank were also added to our dataset (Alpha-CoVs: n = 226 – Beta-CoVs: n = 206).

22

PI: Daszak, Peter

Phylogenetic trees were reconstructed for Alpha- and Beta-CoVs separately using Bayesian inference (BEAST 1.8).
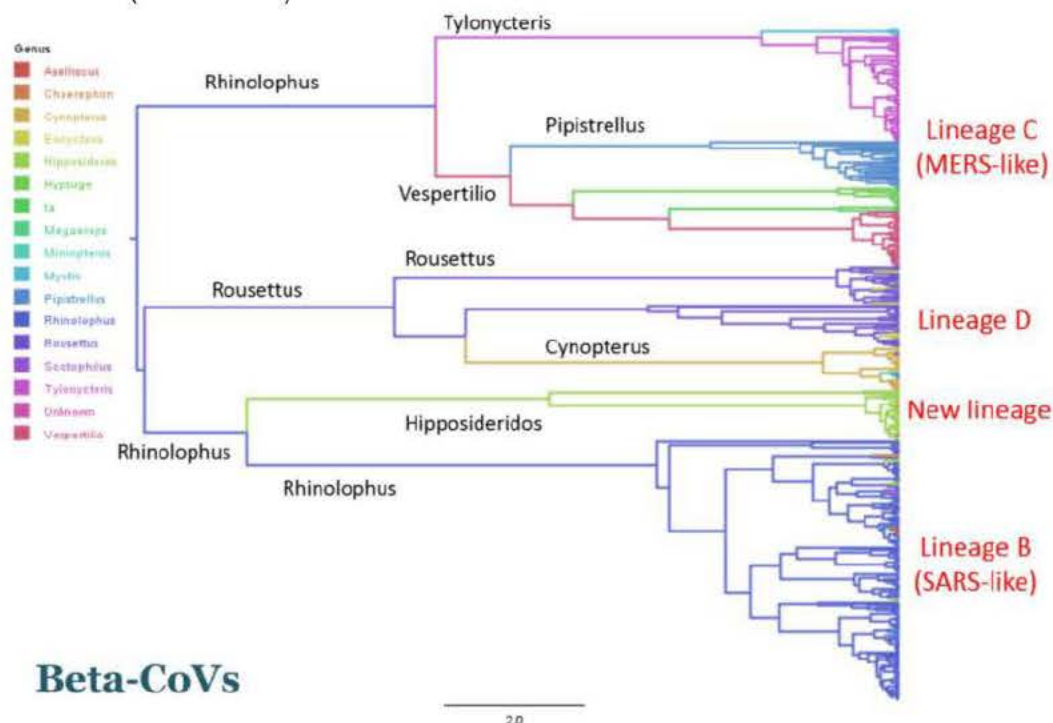


**Figure 31.** Ancestral host reconstruction for Beta-CoVs, at a host genus level.
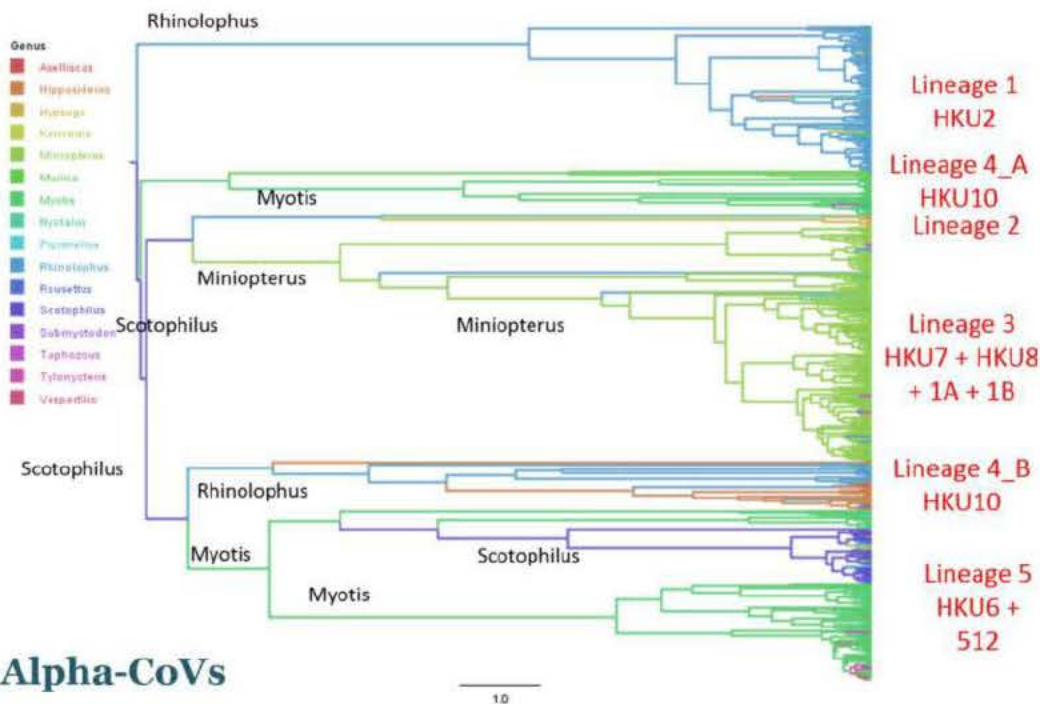


**Figure 32.** Ancestral host reconstruction for Alpha-CoVs, at a host genus level.

To better understand the geographic origins and extent of specific CoV clades, we also used discrete ancestral character state reconstruction in BEAST to reconstruct the ancestral location of each branch of the tree. We used SPREAD to visualize the tree in its geographic context and infer CoV spatial spread in China (**Fig. 33**). These analyses allow us to identify the geographic areas that are likely sources of origin/diversity for this important group of viruses. The common ancestor of most Beta-CoVs lineages is located in Hong Kong and Guangdong. The common ancestor of most Alpha-CoV lineages was located in Yunnan province, and our results suggest they spread to other provinces from Yunnan.



**Beta-CoVs**                          **Alpha-CoVs**
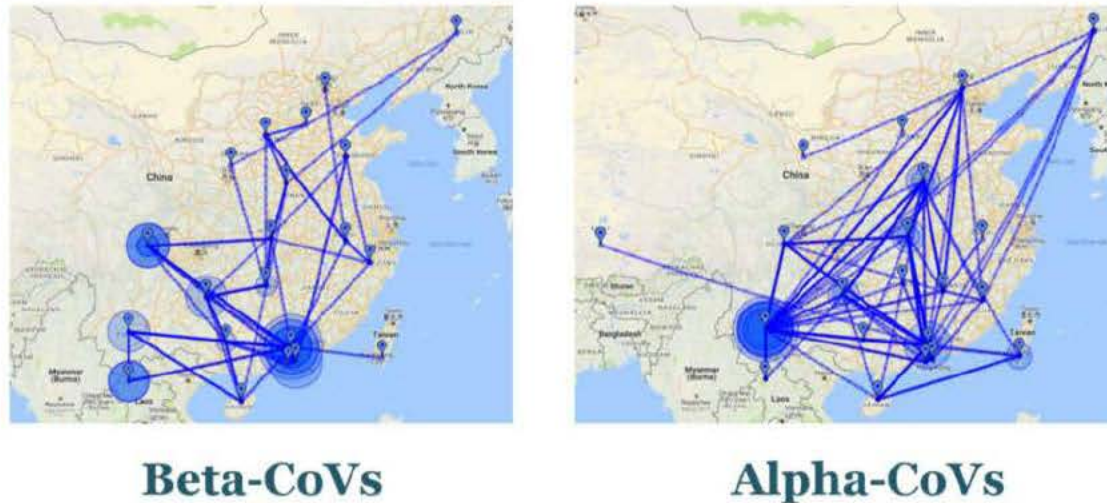
*Figure 33. Ancestral location reconstruction for Beta- and Alpha-CoVs. The bigger the circle is, the more ancestral the corresponding node is.*

## Specific Aim 3: Testing Predictions of CoV Inter-Species Transmission

### Identification of two novel MERS-related CoVs that use DPP4 receptor

Two novel MERSr-CoVs, BtCoV/Ii/GD/2013-845 and BtCoV/Ii/GD/2014-422, were identified from great evening bats (*Ia io*) in Guangdong province. Phylogenetic analysis of polyprotein 1 and the E, M, and N proteins suggests that the two novel strains are more closely related to MERS-CoV than to other lineage C Beta-CoVs. Their RdRp sequences are closely related to those of MERS-CoV and other MERSr-CoVs, with 94.4–97.0% aa identities. In contrast, they are divergent from MERS-CoV and other MERSr-CoVs in the spike protein, with only 58.9–64.7% aa identities. However, in the receptor-binding domain (RBD) of the spike protein, the two novel MERSr-CoVs are identical to MERS-CoV at six out of the 13 residues that directly interact with human DPP4 receptor, making them more similar to MERS-CoV than any other known lineage C BetaCoVs (**Fig. 34a**). Protein–protein interaction assays demonstrated that the spike proteins of the novel MERSr-CoVs bind to both human and bat DPP4 (**Fig. 34b**). Moreover, bat cells exogenously expressing human DPP4 support the entry of the retrovirus pseudotyped with BtCoV/Ii/GD/2014-422 spike, while the pseudovirus fails to enter cells that do not express DPP4. The results demonstrate that the spike protein of the newly identified MERSr-CoV recognizes the human DPP4 receptor.

24

1R01AI110964 Year 4 Report                                          PI: Daszak, Peter

A

```
        467
MERS     FNYKQSFSNP TCLILATVPH NLTT---ITK PLKYSYINKC SRLLSDD-RT   515
422      YNYKQSFANP TCRIFATAPA NLT----ITK PSSYSFISKC SRLTGDNSHI   516
845      FNYKQSFANP TCRIFATAPA NLT----ISK PSSYSYISKC SRLTGDNQHI   517
HKU4     YNYKQSFANP TCRVMASVLA NVT----ITK PHAYGYISKC SRLTGANQDV   517
SC2013   FNYKQDFSNP TCRILATVPA NLSASGLLPK PSNYVWLSEC YQNSFTG---   488
Neo      FNYNQDYSNP SCRIHSKVNS SIG----ISY AGAYSYITNC NYGATNK---   512
PDF-2180 FNYNQDYSNP SCRIHSKVNS SVG----ISY SGLYSYITNC NYGGFNK---   513
HKU5     FNYKQDFSNP TCRVLATVPQ NLTT---ITK PSNYAYLTEC YKTSAYG---   518
         :**:*.::** :* : :..   .:      :.  . * ::.:*          .

        513
MERS     EVPQLVNANQ YSPCVSIVPS TVWEDGDYYR KQLSPLEGGG WLVASGSTVA   562
422      ETPIVINPGE YSICKNFAPN GFSQDGDYFT RQLSQLEGGG ILVGVGSVTP   566
845      ETPITINPGE YSICRGFAPN GLSEDGQVFT RQLSDYEGGG TLVGVGNTVP   567
HKU4     ETPLYINPGE YSICRDFSPG GFSEDGQVFK RTLTQFEGGG LLIGVGTRVP   567
SC2013   KNFQYVKAGQ YTPCLGLAAN GFEKSYQTHR DPV------S KLAVTGVVTP   532
Neo      DDVVKPGGRA SQQCITGALN S-PTTGQLWA YNF-----GG VPYRVSRLTY   556
PDF-2180 DDVVKPGGRA SQPCVTGALN S-PTNGQVWS FNF-----GG VPYRTSRLTY   557
HKU5     KNYLYNAPGA YTPCLSLASR GFSTKYQSHS D--------G ELTTTGYIYP   561
            *           :          .    .
```

B



**Figure 34.** *BtCoV/li/GD/2014-422 RBD analysis (a) and DPP4-binding assay (b)*

### *In Vivo* Infection of Human ACE2 (hACE2) Expressing Mice with SARSr-CoV S Protein variants

Using the reverse genetic methods we previously developed, infectious clones with the WIV1 backbone and the spike protein of SHC014, WIV16 and Rs4231, respectively, were constructed and recombinant viruses were successfully rescued. In Year 4, we performed preliminary *in vivo* infection of SARSr-CoVs on transgenic mice that express hACE2. Mice were infected with $10^5$ pfu of full-length recombinant virus of WIV1 (rWIV1) and the three chimeric viruses with different spikes. Pathogenesis of the 4 SARSr-CoVs was then determined in a 2-week course. Mice challenged with rWIV1-SHC014S have experienced about 20% body weight loss by the 6th day post infection, while rWIV1 and rWIV-4231S produced less body weight loss. In the mice infected with rWIV1-WIV16S, no body weight loss was observed **(Fig. 35a)**. 2 and 4 days post infection, the viral load in lung tissues of mice challenged with rWIV1-SHC014S, rWIV1-WIV16S and rWIV1-Rs4231S reached more than $10^6$ genome copies/g and were significantly higher than that in rWIV1-infected mice **(Fig. 35b)**. These results demonstrate varying pathogenicity of SARSr-CoVs with different spike proteins in humanized mice.

25

**Figure 35.** *In vivo infection of SARSr-CoVs in hACE2-expressing mice. (a, left) Body weight change after infection; (b, right) Viral load in lung tissues*
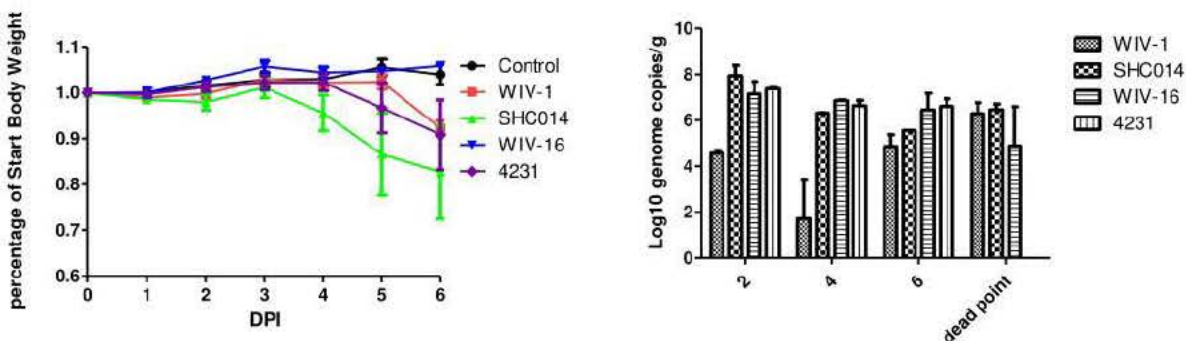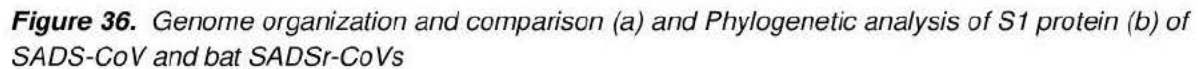
## Additional Year 4 Results for Specific Aim 3:

### Identification of a HKU2-related Coronavirus of Bat Origin that Caused Fatal Acute Diarrhea in Piglets

From October 2016, a series of fatal swine diarrhea disease outbreaks occurred in Guangdong province. By May 2017, it had resulted in death of 24,693 piglets across four farms. We identified a novel coronavirus as the etiological agent of the disease by metagenomic analysis, viral isolation and experimental infection, and named this "Swine Acute Diarrhea Syndrome coronavirus (SADS-CoV). During Year 4, we submitted and published a paper on this finding to *Nature* (Zhou *et al.*, 2018). The full-length genome of SADS-CoV shares 95% sequence identity to bat CoV HKU2. However, the S gene sequence identity is only 86%, suggesting that   the previously reported HKU2-CoV is not the direct progenitor of SADS-CoV, but that they may have originated from a common ancestor.

Using a SADS-CoV specific qPCR assay based on its RdRp gene, SADS-related coronaviruses (SADSr-CoVs) were detected in rectal swabs of *Rhinolophus* bats collected from 2013 to 2016 in Guangdong. Full-length genome sequencing of 4 bat SADSr-CoVs revealed 96% to 98% overall genome sequence identity between SADSr-CoVs and SADS-CoV. Most importantly, the S protein of SADS-CoV shared more than 98% sequence identity with those of the two SADSr-CoVs (162149 and 141388), compared to 86% with HKU2-CoV (**Fig. 36a**). The phylogeny of S1 protein sequence showed strong co-evolutionary relationships with bat alphacoronavirus and their hosts, with swine SADS-CoV more closely related to SADSr-CoVs from *Rhinolophus affinis* than strains from *Rhinolophus sinicus* in which HKU2-CoV was found (**Fig. 36b**). Analysis of the 33 SADS-CoV full genome sequences we were able to characterize from pigs suggests that viruses from the four farms may have been transmitted from their reservoir hosts independently. These findings highlight the importance of identifying coronavirus diversity and distribution in bats to mitigate future outbreaks that threaten livestock and public health.

26

**Figure 36.** *Genome organization and comparison (a) and Phylogenetic analysis of S1 protein (b) of SADS-CoV and bat SADSr-CoVs*

## Intra-Farm Transmission Model to Understand to Predict Future Transmission and Outbreak

To better understand amplification dynamics and assess the potential for future transmission resulting in large outbreaks, we developed an intra-farm, age-structured, stochastic transmission model for SADS-CoV **(Fig. 37)**. We developed multiple versions of this model to represent different hypotheses of disease transmission mechanisms and fit them to time-series data of reported deaths on multiple SADS-infected farms.



$$N = P_S + P_I + P_R + O_S + O_I + O_R + S_S + S_I + S_R$$

$$\Lambda = \beta \frac{(P_I + O_I + S_I)}{N^r}$$

**Figure 37:** *Schematic of intra-farm transmission mode.*

27

Our first model structure, which assumed equal mixing of animals across farms **(Fig. 38)** showed that age structure alone was insufficient to generate the temporal pattern of reported deaths on SADS-infected farms.  Our second model structure **(Fig. 39)** represented individual barns on a farm as a series of pig-virus meta-populations. This structure was sufficient to re-create the dynamics of the series of rapid "mini-epidemics" that progressed in SADS-infected farms.
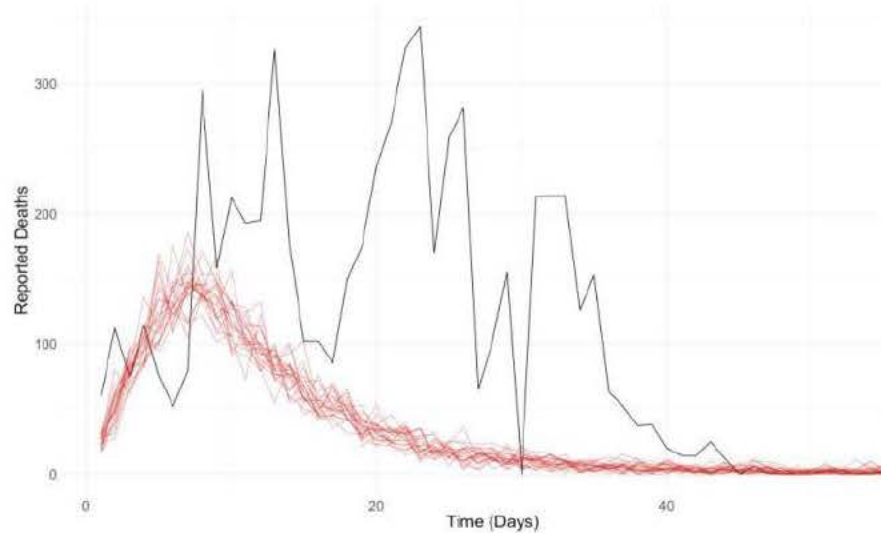


**Figure 38:** *Best-fit simulations (red) from an equal-mixing transmission model and actual reported death time series (black) on a SADS-infected farm.*
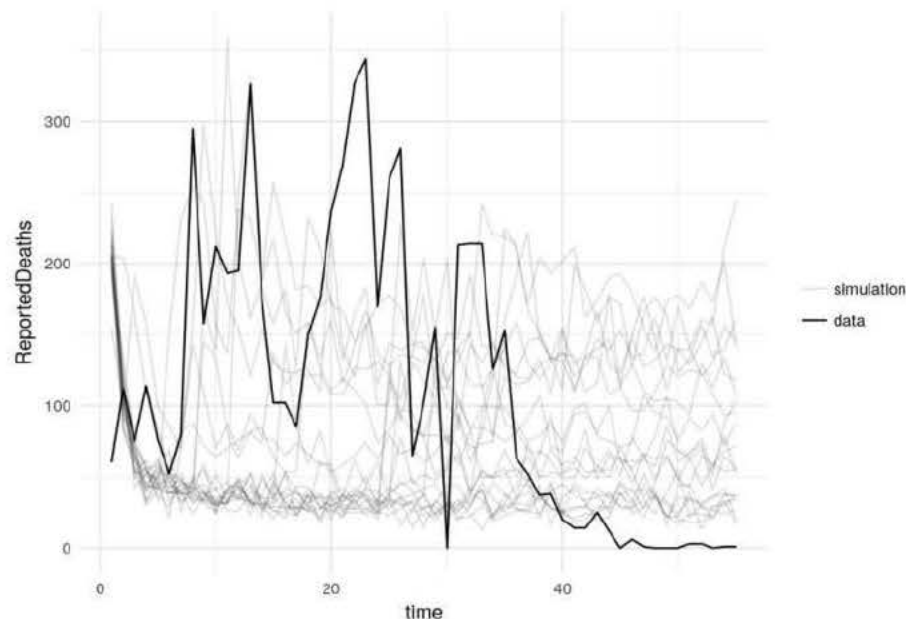


**Figure 39:** *Best-fit simulations (grey) from an metapopulation transmission model and actual reported death time series (black) on a SADS-infected farm.*

28

Specific Goals Not Meet

- The wild animal farm survey was piloted in early Y4, with data collected from seven wild animal farms, it was postponed due to the emergence of SADS-CoV where our group had focused on instead in Y4, but will be resumed in Y5 to continue collecting and analyzing data.
- The passive hospital surveillance has been piloted will continue in Year 4 to collect and test for CoVs.

29

1R01AI110964 Year 4 Report                                    PI: Daszak, Peter

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

1.  Conference and University lectures: We provided human subject research trainings to chief physicians and nurses at local clinics, staff from Yunnan Institute of Endemic Diseases Control and Prevention, students from Dali College and Wuhan University for both qualitative and quantitative research.

2.  Agency and other briefing: Dr. Guangjian Zhu was invited by the Guangdong Institute of Applied Nature Resources, Guangdong Academy of Sciences to provide training to 8 field team members regarding biosafety and PPE use, bats and rodents sampling. Dr. Zhengli Shi participated in the US National Science Foundation-funded EcoHealthNet (grant to EcoHealth Alliance – Epstein PI) that provides research exchange opportunities to undergraduate and graduate-level students.

3.  Public outreach: PI Daszak, and Co-investigators Shi, Epstein, and Olival presented the results of this project to the public via interviews with national central and local television, social media, newspaper and journals in China and the US.

1

## C. PRODUCTS

### C.1 PUBLICATIONS

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

Yes

**Publications Reported for this Reporting Period**

| Public Access Compliance | Citation |
|---|---|
| Complete | Luo CM, Wang N, Yang XL, Liu HZ, Zhang W, Li B, Hu B, Peng C, Geng QB, Zhu GJ, Li F, Shi ZL. Discovery of Novel Bat Coronaviruses in South China That Use the Same Receptor as Middle East Respiratory Syndrome Coronavirus. Journal of virology. 2018 July 1;92(13). PubMed PMID: 29669833; PubMed Central PMCID: PMC6002729; DOI: 10.1128/JVI.00116-18. |
| Complete | Field HE. Evidence of Australian bat lyssavirus infection in diverse Australian bat taxa. Zoonoses and public health. 2018 September;65(6):742-748. PubMed PMID: 29785730; PubMed Central PMCID: PMC6249124; DOI: 10.1111/zph.12480. |
| Complete | Eskew EA, Olival KJ. De-urbanization and Zoonotic Disease Risk. EcoHealth. 2018 December;15(4):707-712. PubMed PMID: 30120670; PubMed Central PMCID: PMC6265062; DOI: 10.1007/s10393-018-1359-9. |
| Complete | Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nature reviews. Microbiology. 2019 March;17(3):181-192. PubMed PMID: 30531947; PubMed Central PMCID: PMC7097006; DOI: 10.1038/s41579-018-0118-9. |
| Complete | Li HY, Zhu GJ, Zhang YZ, Zhang LB, Hagan EA, Martinez S, Chmura AA, Francisco L, Tai H, Miller M, Daszak P. A qualitative study of zoonotic risk factors among rural communities in southern China. International health. 2020 February 12;12(2):77-85. PubMed PMID: 32040190; PubMed Central PMCID: PMC7017878; DOI: 10.1093/inthealth/ihaa001. |

### 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

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? No

### C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

## D. PARTICIPANTS

### D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

| Commons ID | S/K | Name | Degree(s) | Role | Cal | Aca | Sum | Foreign Org | Country | SS |
|---|---|---|---|---|---|---|---|---|---|---|
| (b) (6) | Y | DASZAK, PETER | BS,PHD | PD/PI | | | (b) (4), (b) (6) | | | NA |
| (b) (6) | N | Chmura, Aleksei | BS,PHD | Non-Student Research Assistant | | | | | | NA |
| (b) (6) | N | Ross, Noam Martin | PhD | Co-Investigator | | | | | | NA |
| (b) (6) | Y | Olival, Kevin J. | PHD | Co-Investigator | | | | | | NA |
| (b) (6) | Y | Zhang, Shu-yi | PHD | Co-Investigator | | | | East China Normal University | CHINA | NA |
| | N | ZHU, GUANGJIAN | PHD | Co-Investigator | | | | East China Normal University | CHINA | NA |
| | N | GE, XINGYI | PHD | Co-Investigator | | | | Wuhan Institute of Virology | CHINA | NA |
| | N | KE, CHANGWEN | PHD | Co-Investigator | | | | Center for Disease Control and Prevention of Guangdong Province | CHINA | NA |
| | Y | ZHANG, YUNZHI | PHD | Co-Investigator | | | | Yunnan Provincial Institute of Endemic Diseases Control & Prevention | CHINA | NA |
| (b) (6) | N | EPSTEIN, JONATHAN H | MPH,DVM,BA,PHD | Co-Investigator | | | | | | NA |
| (b) (6) | N | SHI, ZHENGLI | PhD | Co-Investigator | | | | Wuhan Institute of Virology | CHINA | NA |

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

Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

### D.2 PERSONNEL UPDATES

#### D.2.a Level of Effort

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

No

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

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

No

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

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

No

### D.2.d New Other Significant Contributors

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

No

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

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

NA

## E. IMPACT

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

Not Applicable

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

NOTHING TO REPORT

**E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?**

Not Applicable

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

| Dollar Amount | Country |
|---------------|---------|
| $201,422 | 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

| Sub-Project ID: | Study ID | Study Title: | Delayed Onset | Clinical Trial | NCT | NIH-Defined Phase 3 | ACT |
|---|---|---|---|---|---|---|---|
| | 58010 | Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001 | NO | NO | | NO | |

### G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

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

No

### G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

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

No

### G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

### G.8 PROJECT/PERFORMANCE SITES

| Organization Name: | DUNS | Congressional District | Address |
|---|---|---|---|
| Primary: EcoHealth Alliance, Inc. | 077090066 | NY-010 | 460 West 34th Street 17th Floor New York NY 100012317 |
| Wuhan Institute of Virology | 529027474 | | Xiao Hong Shan, No. 44 Wuchang District Wuhan NONE |
| East China Normal University | 420945495 | | 3663 Zhongshan Beilu Shanghai NONE |
| ECOHEALTH ALLIANCE | 077090066 | | ECOHEALTH ALLIANCE, INC. 460 W 34TH ST |

| | | | NEW YORK NY 100012320 |
|---|---|---|---|

## G.9 FOREIGN COMPONENT

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

**Organization Name:** Wuhan School of Public Health
**Country:** CHINA
**Description of Foreign Component:**
Principal Coordinating Team for all project field work as per section G8 (above) and detailed in our Specific Aims

## G.10 ESTIMATED UNOBLIGATED BALANCE

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

No

## G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

## G.12 F&A COSTS

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

No

## Section 1 - Basic Information (Study 58010)

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

1.1. Study Title *

Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

1.2. Is this study exempt from Federal Regulations *      ○ Yes      ● No

1.3. Exemption Number      ❑ 1      ❑ 2      ❑ 3      ❑ 4      ❑ 5      ❑ 6      ❑ 7      ❑ 8

1.4. Clinical Trial Questionnaire *

   1.4.a. Does the study involve human participants?      ● Yes      ○ No

   1.4.b. Are the participants prospectively assigned to an intervention?      ○ Yes      ● No

   1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?      ○ Yes      ● No

   1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?      ○ Yes      ● No

1.5. Provide the ClinicalTrials.gov Identifier (e.g. NCT87654321) for this trial, if applicable

## Section 2 - Study Population Characteristics (Study 58010)

2.1. Conditions or Focus of Study

2.2. Eligibility Criteria

2.3. Age Limits                           Min Age:                    Max Age:

2.4. Inclusion of Women, Minorities, and Children

2.5. Recruitment and Retention Plan

2.6. Recruitment Status              Not yet recruiting

2.7. Study Timeline

## Section 2 - Study Population Characteristics (Study 58010)

**Inclusion Enrollment Reports**

| IER ID# | Enrollment Location Type | Enrollment Location |
|---------|--------------------------|---------------------|
| IER 58010 | Foreign | |

## Inclusion Enrollment Report 58010

Using an Existing Dataset or Resource* :　　　○ Yes　　　● No

Enrollment Location Type* :　　　○ Domestic　　● Foreign

Enrollment Country(ies):　　　CHN: CHINA

Enrollment Location(s):

Comments:

**Planned**

| Racial Categories | Ethnic Categories | | | | Total |
|---|---|---|---|---|---|
| | Not Hispanic or Latino | | Hispanic or Latino | | |
| | Female | Male | Female | Male | |
| American Indian/ Alaska Native | 0 | 0 | 0 | 0 | 0 |
| Asian | 1230 | 1230 | 0 | 0 | 2460 |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 | 0 | 0 |
| Black or African American | 0 | 0 | 0 | 0 | 0 |
| White | 0 | 0 | 0 | 0 | 0 |
| More than One Race | 0 | 0 | 0 | 0 | 0 |
| Total | 1230 | 1230 | 0 | 0 | 2460 |

**Cumulative (Actual)**

| Racial Categories | Ethnic Categories | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | Not Hispanic or Latino | | | Hispanic or Latino | | | Unknown/Not Reported Ethnicity | | | |
| | Female | Male | Unknown/ Not Reported | Female | Male | Unknown/ Not Reported | Female | Male | Unknown/ Not Reported | |
| American Indian/ Alaska Native | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Asian | 980 | 616 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1596 |
| 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 | 980 | 616 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1596 |

## Section 3 - Protection and Monitoring Plans (Study 58010)

3.1. Protection of Human Subjects

3.2. Is this a multi-site study that will use the same protocol to     ○ Yes     ○ No     ○ N/A
conduct non-exempt human subjects research at more than one
domestic site?

      If yes, describe the single IRB plan

3.3. Data and Safety Monitoring Plan

3.4. Will a Data and Safety Monitoring Board be appointed for     ○ Yes     ○ No
this study?

3.5. Overall structure of the study team

## Section 4 - Protocol Synopsis (Study 58010)

4.1. Brief Summary

4.2. Study Design

    4.2.a. Narrative Study Description

    4.2.b. Primary Purpose

    4.2.c. Interventions

| Type | Name | Description |
|------|------|-------------|
|      |      |             |

    4.2.d. Study Phase

        Is this an NIH-defined Phase III Clinical Trial?    ○ Yes    ● No

    4.2.e. Intervention Model

    4.2.f. Masking    ○ Yes    ○ No

        ❏ Participant    ❏ Care Provider    ❏ Investigator    ❏ Outcomes Assessor

    4.2.g. Allocation

4.3. Outcome Measures

| Type | Name | Time Frame | Brief Description |
|------|------|------------|-------------------|
|      |      |            |                   |

4.4. Statistical Design and Power

4.5. Subject Participation Duration

4.6. Will the study use an FDA-regulated intervention?    ○ Yes    ○ No

    4.6.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/ Investigational Device Exemption (IDE) status

4.7. Dissemination Plan