

Wuhan Final Interim Report

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: 06/01/2018 - 05/31/2019	Requested Budget Period: 06/01/2018 - 05/31/2019
Report Term Frequency: Annual	Date Submitted: 08/03/2021
Program Director/Principal Investigator Information: PETER DASZAK, PHD BS Phone Number: (b) (6) Email: (b) (6)	Recipient Organization: ECOHEALTH ALLIANCE, INC. ECOHEALTH ALLIANCE, INC. 520 EIGHTH AVENUE NEW YORK, NY 100181620 DUNS: 077090066 EIN: 1311726494A1 RECIPIENT ID:
Change of Contact PD/PI: NA	
Administrative Official: ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 Phone number: (b) (6) Email: (b) (6)	Signing Official: ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 Phone number: (b) (6) Email: (b) (6)
Human Subjects: Yes HS Exempt: NA Exemption Number: Phase III Clinical Trial: NA	Vertebrate Animals: NA
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.

F 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 5 NIAID CoV Report Accomplishments Final.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 : B4 Training.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 conference and university lectures at The US-China Dialogue on the Challenges of Emerging Infections, Laboratory Safety and Global Health Security in Galveston, US; the US-China Workshop on Frontiers in Ecology and Evolution of Infectious Diseases in Berkeley, US and Shenzhen, China; the Sino-Germany symposium "Globalization-Challenge and Response for Infectious Diseases" in Hamburg, Germany; the 8th International Symposium on Emerging Viral Diseases in Wuhan, China; the Global Virome Project meeting, Bangkok, Thailand; the Western Asia Bat Research Network (WAB-Net) workshop, Tbilisi, Georgia; the International Conference on Emerging Infectious Diseases (ICEID), Atlanta, US; the North American Society for Bat Research (NASBR) Conference, Puerto Vallarta, Mexico; and the 3rd Symposium of Biodiversity and Health in Southeast Asia, Chiayi, Taiwan

2. Agency and other briefing: PI Daszak and Co-investigators Shi, Olival presented this project at the Cary Institute for Ecosystem Studies, New York, US; the National Institute for Viral Disease Control and Prevention, China CDC; the Chinese Academy of Sciences; and the Chinese Academy of Medical Sciences

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 5 including at a Cosmos Club briefing that EcoHealth Alliances hosts in Washington DC, multiple meetings of the China National Virome Project and the Global Virome Project in China, Europe, Australia, Southeast Asia and Latin America. As in Year 4, Co-Investigator Zhu 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?

Not Applicable

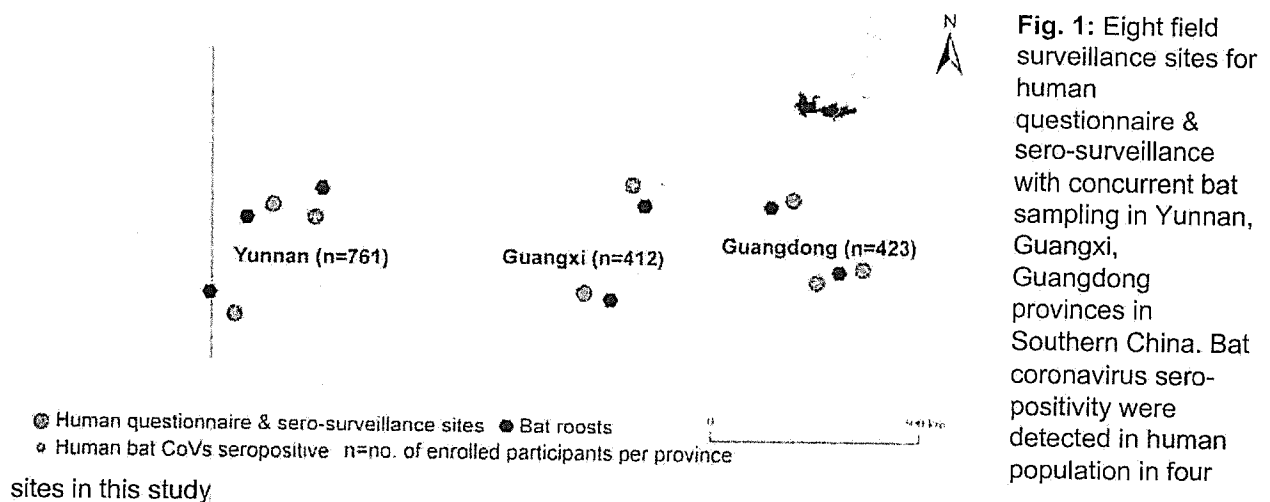
The results of the 5th year of our R01 work are detailed below. They include:

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

During Year 5, we finalized the analysis of both quantitative and qualitative data from human surveillance in three provinces in Southern China: Yunnan, Guangxi, and Guangdong provinces.

1.1 High-risk human-animal interaction increase bat coronavirus spillover potential among rural residents in southern China

We conducted a cross-sectional biological behavioral surveillance in Yunnan, Guangxi, and Guangdong provinces from 2015 to 2017. From 8 study sites, a total of 1,596 residents were enrolled, of these, 1,585 participants completed the questionnaires and 11 participants withdrew from the questionnaire interview due to personal schedule reasons. After the interviews, 1,497 participants provided biological samples for lab analysis (**Fig. 1**).



1.1.1 Demographics

There were more female (62%) than male (38%) from the communities participated in this study. Most participants were adults over 45 years old (69%) and had been living in the community for more than 5 years (97%) with their family members (95%). A majority relied on a comparatively low family annual per capita income less than 10,000 RMB (86%), which is below the national level of per capita disposable income of rural households from 2015 to 2017. Most participants (98%) had not received a higher education from college and were making a living on crop production (76%). 9% of the participants frequently traveled outside the county as migrant laborers. Some participants were working in sectors where frequent human-animal contacts occur, such as the animal production business (1.7%), wild animal trade (0.5%), slaughterhouses or abattoirs (0.5%), protected nature reserve rangers (0.4%) or in wildlife restaurants (0.3%). It was common for participants to have multiple part-time jobs as income sources (**Table 1**).

Variable	Total	
	N	Valid %
Gender (n= 1,574)		
Female	968	61.5
Male	605	38.4
Other	1	0.1
Age (n=1,582)		
Under 18 years	71	4.5
18 to 44 years	420	26.5
45 to 64 years	780	49.3
Age 65 or older	311	19.7
Province (n=1,585)		
Guang Dong	420	26.5
Guang X	412	26.0
Yun Nan	753	47.5
Time of residence (n=1,568)		
< 1 month	4	0.3
1 month – 1 year	12	0.8
1 year – 5 years	26	1.7
> 5 years	1,526	97.3
Family annual per capita income (RMB) (n=1,565)		
<1000	271	17.3
1001-10000	1067	68.2
>10000	227	14.5
Activities to earn livelihood since last year		
Extraction of minerals, gas, oil, timber (n=1,566)	5	0.3
Crop production (n=1,569)	1,196	76.2
Welfare restaurant business (n=1,564)	5	0.3
Wholesale/retail trade/market business (n=1,566)	8	0.5
Rancher/farmer animal product business (n=1,566)	27	1.7
Meat processing, slaughterhouse, abattoir (n=1,567)	8	0.5
Zoo/sanctuary animal health care (n=1,565)	1	0.1
Protected area worker (n=1,567)	7	0.4
Hunter/trapper/fisher (n=1,565)	3	0.2
Forager/gatherer/non-timber forest product collector (n=1,566)	4	0.3
Migrant laborer (n=1,567)	144	9.2
Nurse, doctor, healer, community health worker (n=1,567)	7	0.4
Construction (n=1,564)	41	2.6
Other (n=1,568)	293	18.7
Highest level of education you completed (n=1,570)		
None	428	27.3
Primary School	632	40.3
Secondary school /Polytechnic school	479	30.5
College/university/professional	31	2.0
Live with family (n=1,564)		
No	73	4.7
Yes	1491	95.3

Table 1: Demographics of study participants. Total counts differ due to missing responses.

1.1.2 Animal contact and exposure to bat coronaviruses

Serological testing of serum samples from 1,497 local residents revealed 9 individuals (0.6%) were positive for bat coronavirus, indicating exposure at any point in their life to bat-born SARS-related Coronavirus (n=7, Yunnan) and HKU10 Coronavirus (n=2, Guangxi), or other coronaviruses that are phylogenetically closely related to these two coronaviruses (Table 2). All individuals who tested positive (male=6, female=3) were over 45 years old, and most (n=8)

were making a living from crop production. None of those participants reported any symptoms in the preceding 12 months in the interview.

Site	# tested	Bat CoV + (%)	SARSr-CoV Rp3 + (%)	HKU10 + (%)	HKU9 + (%)	MERS-CoV+ (%)
J n n ng, Yunnan	209	6 (2.87)	6 (2.87)	-	-	-
Meng a, Yunnan	168	1 (0.6)	1 (0.6)	-	-	-
J nghong, Yunnan	212	-	-	-	-	-
Lufeng, Yunnan	144	-	-	-	-	-
Guangdong	420	-	-	-	-	-
Guangx	412	2 (0.48)	-	2 (0.48)	-	-

Table 2: ELISA testing of human sera for 4 bat CoVs

Due to the low rate of sero-positivity, we did not conduct statistical comparisons of animal-contact behavior by coronavirus outcome. Figure 2 shows animal contact rates among the survey population (n= 1,585) and among sero-positive individuals (n=9). Participants reported common contact with poultry and rodents/shrews, and most animal contact occurred in domestic settings through raising animal or food preparation activities.

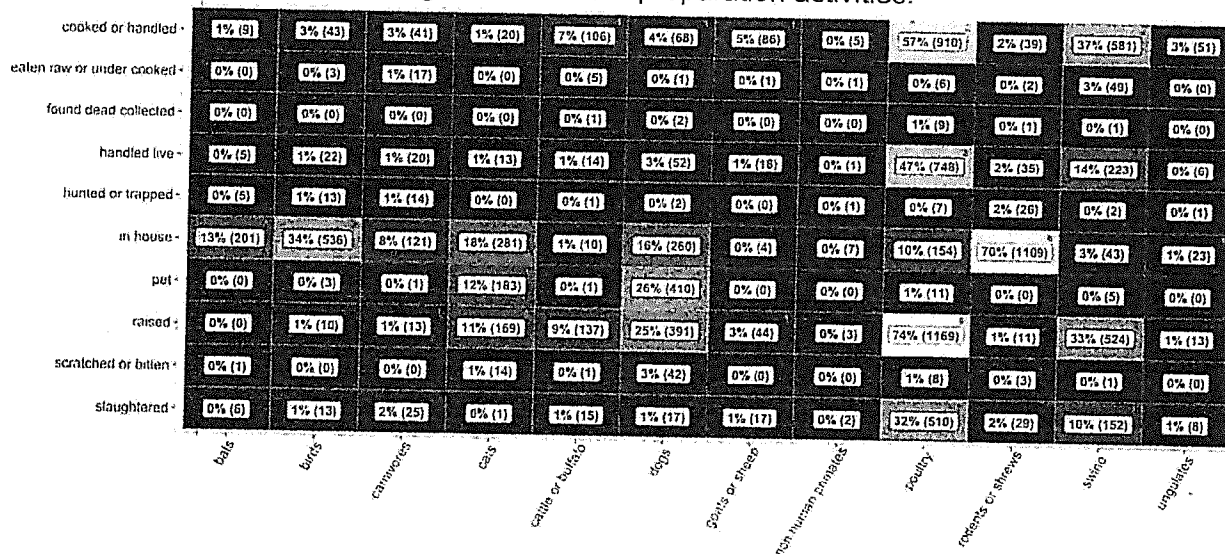


Fig. 2: Animal contact by taxa and activities. Values and shading represent survey population; red numbers in upper-right corners of cells indicate the number of sero-positive individuals with the given contact.

1.1.3 Self-report SARI/ILI symptoms and animal contact

Among the 1,565 participants who responded, 17% (n=265) had experienced fever with cough and shortness of breath or difficulty breathing (38, 14%), indicative of severe acute respiratory infection (SARI), or fever with muscle aches; cough, or sore throat (192, 72%), indicative of influenza like illness (ILI), or both symptoms (35, 13%) in the past 12 months.

LASSO analyses of the associations between animal contact and self-report SARI or ILI symptoms showed that eating raw or undercooked carnivores (OR = 1.6; bootstrap support = 0.67) was the most salient predictor of experiencing SARI or ILI symptoms, followed by slaughtering poultry as a resident of Guangxi province (OR = 1.4; support = 0.68); having an income below 10,000 as a resident of Guangxi province (OR = 1.3; support = 0.84); domestic

contact with bats (OR = 1.3 ; support = 0.63) and domestic contact with rodents or shrews as a resident of Guangdong province (OR = 1.2; support = 0.63) (**Fig. 3**).

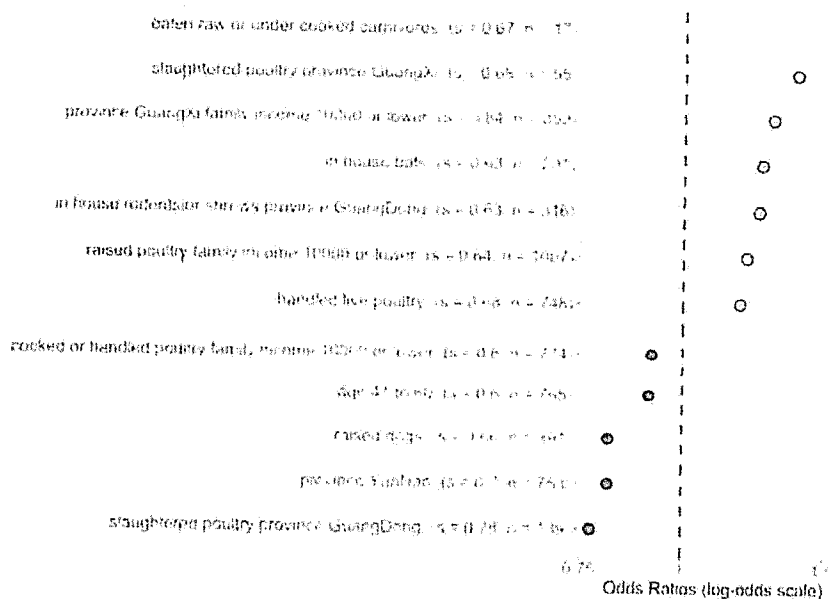


Fig. 3: Most salient predictors of self-reported ILI and/or SARI symptoms in the last year (s = bootstrap support; n = count positive out of 1585 respondents). Bootstrap support values = 0.6 are demonstrated here meaning they were identified as associated with the outcome for 60% or more of the bootstrap iterations. Odds ratios > 1 (orange) are positively associated with the outcome, and odds ratios < 1 (purple) are negatively associated with the outcome.

This study provides serological evidence of subclinical or asymptomatic bat-born SARS-related Coronavirus and HKU10 Coronavirus spillover event(s) in rural communities in Southern China, highlights the associations between human-animal interaction and zoonotic spillover risk. The rate of seropositivity observed in this study is clearly lower than would be seen for established human infections. However it has important implications for predicting and preventing pandemics:

1. It indicates that spillover of novel bat-CoVs is detectable if populations that live within areas inhabited by likely bats hosts are targeted. **This provides a pathway to identify spillover events rapidly, perhaps even before a SARS-like disease can become established in people;**
2. It allows us to calculate the likely number of people infected by novel bat SARSr-CoVs annually in this region. Our preliminary analyses suggest that if similar seroprevalence occurs in human populations across the region bat SARSr-CoV hosts inhabit, **there may be as many as the low hundreds of thousands to over a million people infected each year in South China and Southeast Asia.** We aim to conduct a detailed analysis of this in the future.
3. It highlights ways to refine surveillance that could help prevent pandemics, by targeting populations where seroprevalence suggests that they are **at higher risk due to behavioral preferences (e.g. wildlife hunting, farming, or trading) or where early-stage SARS-like illnesses could be identified using syndromic surveillance of clinics.**

Contact with poultry and rodents/shrews were commonly reported among participants and associated with self-reported ILI and/or SARI symptoms, which suggests that domestic animals, in addition to wildlife, are an important link in understanding the coronavirus transmission from bat to human populations, indirect exposure might occur through contact with live domestic animals in house or market when the animals had prior exposure to bat coronavirus.

When clinical evidence is limited, undiagnosed or subclinical symptoms similar to SARI and ILI in a population should be brought to our attention as indicators in monitoring zoonotic pathogen spillover events, and considered for prevention strategies. This is particularly important in rural community settings, where people have a higher level of exposure to both domestic and wild animals, but may not seek diagnosis or treatment in a timely fashion, thus slowing the processes of early detection and response.

1.2 Qualitative Approach to Developing Zoonotic Risk Mitigation Strategies in Southern China

To explore the potential drivers of zoonotic exposure and the opportunities for intervention, we conducted field observation and semi-structured ethnographic interviews among 88 community members who have frequent exposure to wildlife and domestic animals and/or have extensive local knowledge in 9 sites in Yunnan, Guangdong, and Guangxi provinces.

The majority of participants in this study were adults between 31 to 50 years of age, residing in rural or suburban areas. Most earned their livelihoods from multiple sources, primarily in crop production, subsistence animal farming, small business, and other temporary jobs as migrant workers. Risk and protective factors were identified at the individual, community, and policy levels regarding potential zoonosis exposures, recommending risk-mitigation strategies with the strengthened policy enforcement and multi-sectoral collaboration among human, animal, and environment health programs (Fig. 4).

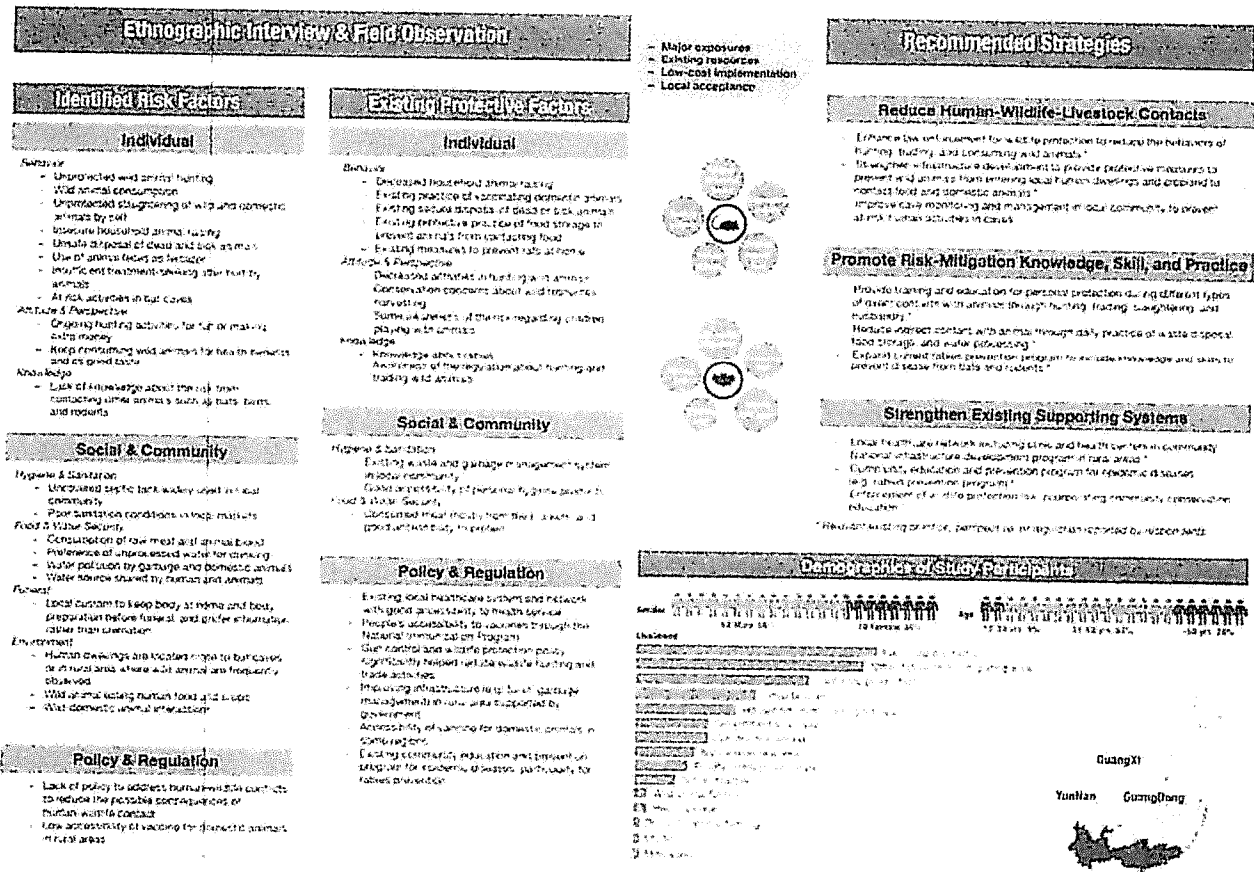


Fig. 1: Community Zoonosis Exposure Risk Mitigation Strategy Development Process. Leveraging ethnographic interview and observational research data to identify risk and protective factors and develop risk-mitigation recommendations

This demonstrated a qualitative approach to understand the zoonotic risks in community, and provided guidance for future research and interventions with focused potential zoonotic risks for disease control and prevention in southern China and a broader area with similar ecological, culture, and demographic contexts.

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

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

From May to October 2018, we collected 1,697 rectal swabs, oral swabs, and feces specimens from 26 bat species in Hubei, Shandong, Yunnan and Guangdong Provinces across southern, central and northern China in Year 5, all specimen were tested for CoV RNA and 109 (6.4%) were positive. SARS-related coronaviruses were discovered in *Rhinolophus sinicus* samples from Yunnan and Hubei provinces while HKU2-related coronaviruses were detected in *R. sinicus* from Hubei. HKU5-related and HKU10-related coronaviruses were identified in *Pipistrellus abramus* from Shandong and *Hipposideros larvatus* from Guangdong, respectively. *Scotophilus* coronavirus 512 was detected in Guangdong. Additionally, two novel *Pipistrellus* alphacoronaviruses were found in Shandong province in northern China (**Fig. 5**).

Fig. 2: Phylogenetic analysis of partial RdRp gene of CoV (440-nt partial sequence)

2.2 Bat coronavirus host-virus phylogeography in China

Our dataset includes all CoV RdRp sequences isolated from bat specimens collected by our team from 2008-2015 (Alpha-CoVs: n = 491 – Beta-CoVs: n = 326), including those collected under prior NIAID funding (1 R01 AI079231), and funding from Chinese Federal Agencies. All Chinese bat CoV RdRp sequences available in GenBank were also added to

our dataset (Alpha-CoVs: $n = 226$ – Beta-CoVs: $n = 206$). Phylogenetic trees were reconstructed for Alpha- and Beta-CoVs separately using Bayesian inference (BEAST 1.8).

2.2.1 Ancestral hosts and cross-species transmission

We used ancestral character state reconstruction and a Bayesian stochastic search variable selection (BSSVS) to identify host switches between bat families (Fig. 6) and genera (Fig. 7) that occurred along the branches of the phylogenetic tree and calculated BF to estimate the significance of these non-zero transition rates. We identified nine and three highly supported ($BF > 10$) **inter-family** host transition rates for alpha- and beta-CoVs, respectively (Figs. 6A and 6B). To quantify the intensity of these host switches, we estimated the number of state changes (Markov jumps) along the significant inter-family transition rates (Figs. 6C and 6D). The total estimated number of inter-family host jump events was more than eight times higher in the evolutionary history of alpha- ($n = 90$) than beta-CoVs ($n = 11$) in China. Host transition events from Rhinolophidae and Miniopteridae were greater than from other families for alpha-CoVs while Rhinolophidae were the highest donor family for beta-CoVs. Rhinolophidae and Hipposideridae were the families receiving the highest numbers of transition events for alpha- and beta-CoVs, respectively (Figs. 6C and 6D).

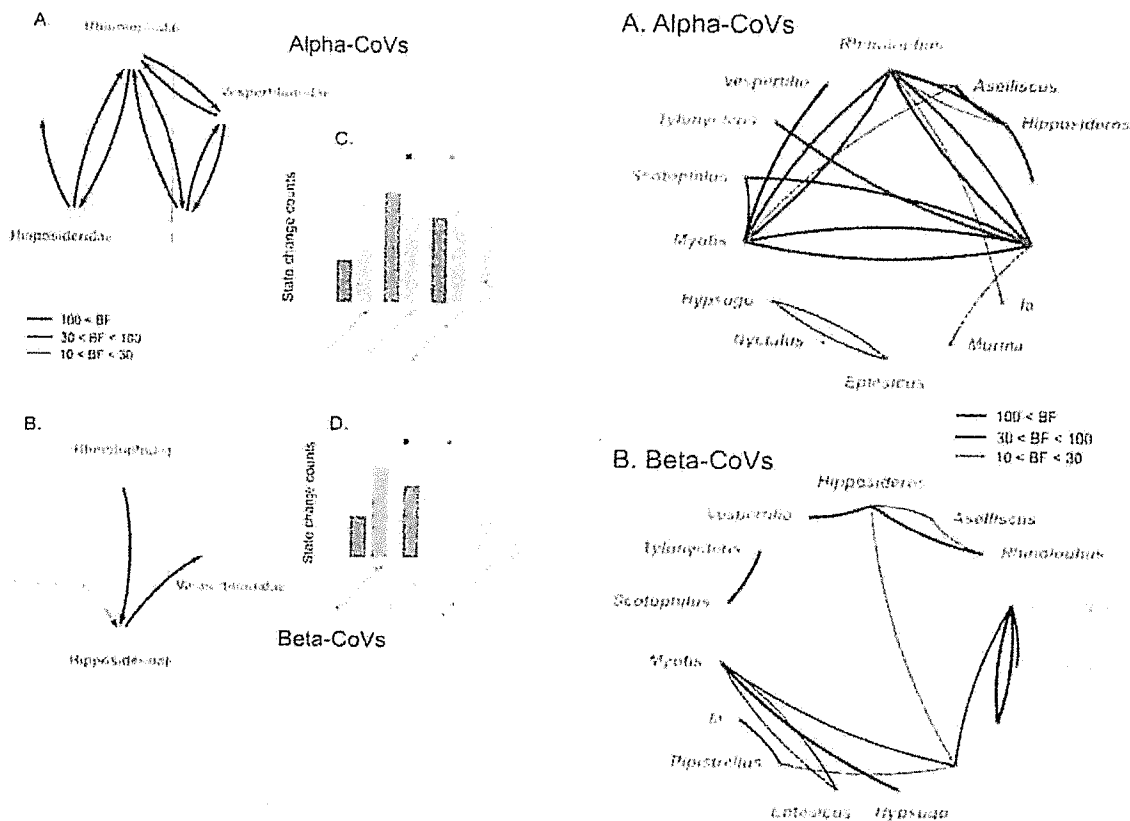


Figure 3: Non-zero transition rates between bat families for alpha- (A) and beta-CoVs (B) and their significance level (Bayes factor, BF), $BF < 10$ are considered as non-significant. Arrows indicate the direction of the transition; arrow thickness is proportional to the transition significance level. Histograms show total number of state changes (Markov jumps) from/to each bat family along the significant inter-family transition rates for alpha- (C) and beta-CoVs (D).

Figure 4: Non-zero transition rates between bat genera for alpha- (A) and beta-CoVs (B) and their significance level (Bayes factor, BF), BF < 10 are considered as non-significant. Lines with a rightward curvature depict transitions from that bat genus, while lines with leftward curvature depict transition to that bat genus. Inter-family transitions are highlighted in red.

At the genus level, we identified 20 highly supported inter-genus host transition rates for alpha-CoVs (Fig. 7A). *Rhinolophus* and *Myotis* were the donor genera in four of these transitions while *Miniopterus* and *Rhinolophus* were each the recipients of four of these transitions (Fig. 7A). Sixteen highly supported inter-genus transition rates were identified for beta-CoVs (Fig. 7B). Four of these 16 host switches originated in *Cynopterus* while three of them ended in *Myotis* (Fig. 7B). Fifteen out of the 20 significant pairwise host transitions (75%) for alpha-CoVs involved two genera belonging to different bat families, while this proportion is only 6/16 (37.5%) for beta-CoVs. This confirmed the highest number of inter-family host transitions for alpha-CoVs. The estimated total number of inter-genus host switches was almost two times higher for alpha- ($n = 123$) than beta-CoVs ($n = 70$).

These findings indicate that alpha-CoVs were able to switch hosts more frequently and between more distantly related taxa during their evolution and suggest that phylogenetic distance among hosts represents higher constraint on host switches for beta- than alpha-CoVs.

2.2.2 CoV spatiotemporal dispersal in China

We also used our Bayesian discrete phylogeographic model using zoogeographic regions as character states to reconstruct the spatiotemporal dynamics of CoV dispersal in China. Eleven and seven highly significant (BF > 10) dispersal routes within China were identified for alpha- and beta-CoVs, respectively (Fig. 8A and 8B). The Rhinacovirus lineage that includes HKU2 and SADS-CoV likely originated in SO region while all other alpha-CoV lineages likely arose in SW China and spread to other regions before several dispersal events occurred from SO and NO in all directions (Fig. 8A).

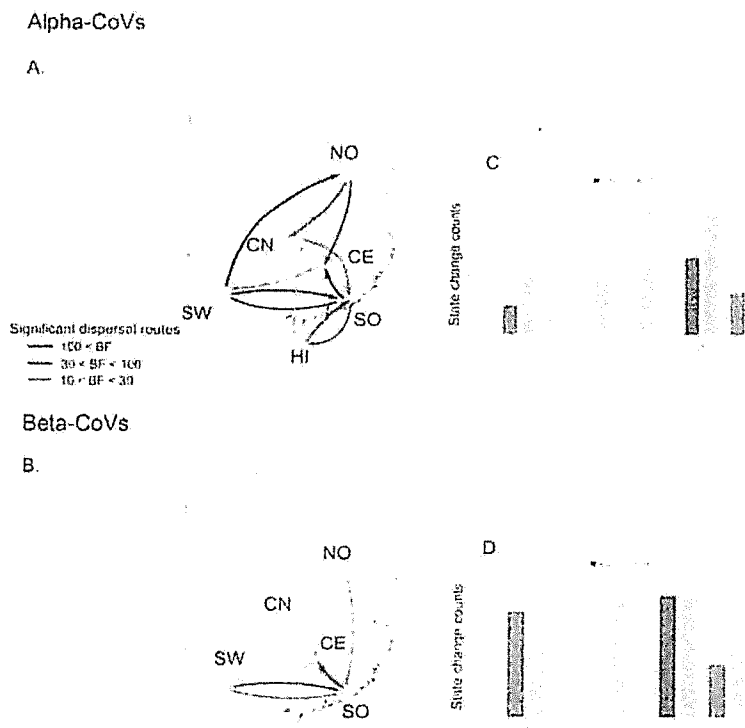


Fig. 8: Significant dispersal routes among China zoogeographic regions for alpha- (A) and beta-CoVs (B). Arrows indicate the direction of the transition; arrow thickness is proportional to the transition significance level. Darker arrow colors indicate older dispersal events. Fig. 8 (C & D) Histograms of total number of state changes (Markov jumps) from/to each region along the significant dispersal routes for alpha- (C) and beta-CoVs (D). NO, Northern region; CN, Central northern region; SW, South western region; CE, Central region; SO, Southern region; HI, Hainan island.

The oldest inferred dispersal movements among beta-CoVs occurred among SO and SW regions (Fig. 8B). SO region is the likely origin of Merbecovirus (Lineage C, including HKU4 and

HKU5) and Sarbecovirus subgenera (Lineage B, including HKU 3 and SARS-related CoVs) while Nobecovirus (lineage D) and Hibecovirus (lineage E) subgenera originated in SW China. Then several dispersal movements likely originated from SO and CE (**Fig. 8B**). More recent southward dispersal from NO was observed.

The estimated total number of migration events along these significant dispersal routes is four times higher for alpha- ($n = 227$) than beta-CoVs ($n = 57$). SO has the highest number of outbound and inbound migration events for alpha-CoVs (**Fig. 8C**). For beta-CoVs, the highest numbers of outbound migration events have been estimated from NO and SO while SO and SW have the highest numbers of inbound migration events (**Fig. 8D**).

Our Bayesian ancestral reconstructions revealed the high importance of South western and Southern China as centers of diversification for both alpha- and beta-CoVs. These two regions are clearly hotspots of CoV phylo-diversity, harboring evolutionary old and phylogenetically diverse lineages of alpha- and beta- CoVs.

2.2.3 Phylogenetic diversity

In order to quantitatively evaluate the diversity and the clustering process in our phylogenies, the Mean Phylogenetic Distance (MPD) and the Mean Nearest Taxon Distance (MNTD) statistics and their standardized effect size (SES) were calculated for each zoogeographic region, bat family and genus. The SES corresponds to the difference between the phylogenetic distances in the observed communities versus null communities built by randomly reshuffling tip labels 1000 times along the entire phylogeny. Low and negative SES values denote phylogenetic clustering, high and positive values indicate phylogenetic over-dispersion while values close to 0 show random dispersion.

Significant negative SES MPD values ($p < 0.05$), indicating basal phylogenetic clustering, were observed within all bat families and genera for both alpha- and beta-CoVs, except within *Aselliscus* and *Tylonycteris* for alpha-CoVs (**Figs. 9A & B**). Negative and mostly significant SES MNTD values, reflecting phylogenetic structure closer to the tips, were also observed within most bat families and genera for alpha- and beta-CoVs but we found non-significant positive SES MNTD value for Vespertilionidae and *Pipistrellus* for beta-CoVs (Fig. 4A and 4B). In general, we observed lower phylogenetic diversity for beta- than alpha-CoVs within all bat families and most genera when looking at SES MPD, while similar level of diversity are observed when looking at SES MNTD (**Figs. 9A & B**). These results suggest stronger basal clustering (at the deeper nodes) for beta-CoVs than alpha-CoVs.

Chinese zoogeographic regions don't harbor a random set of CoVs as alpha- and beta-CoV strains within most regions are more closely related than expected by chance as denoted by negative and mostly significant values of MPD and MNTD (**Fig. 9C**). However, positive SES MPD value for alpha-CoVs in SW indicate wider evolutionary diversity in that region (**Fig. 9C**).

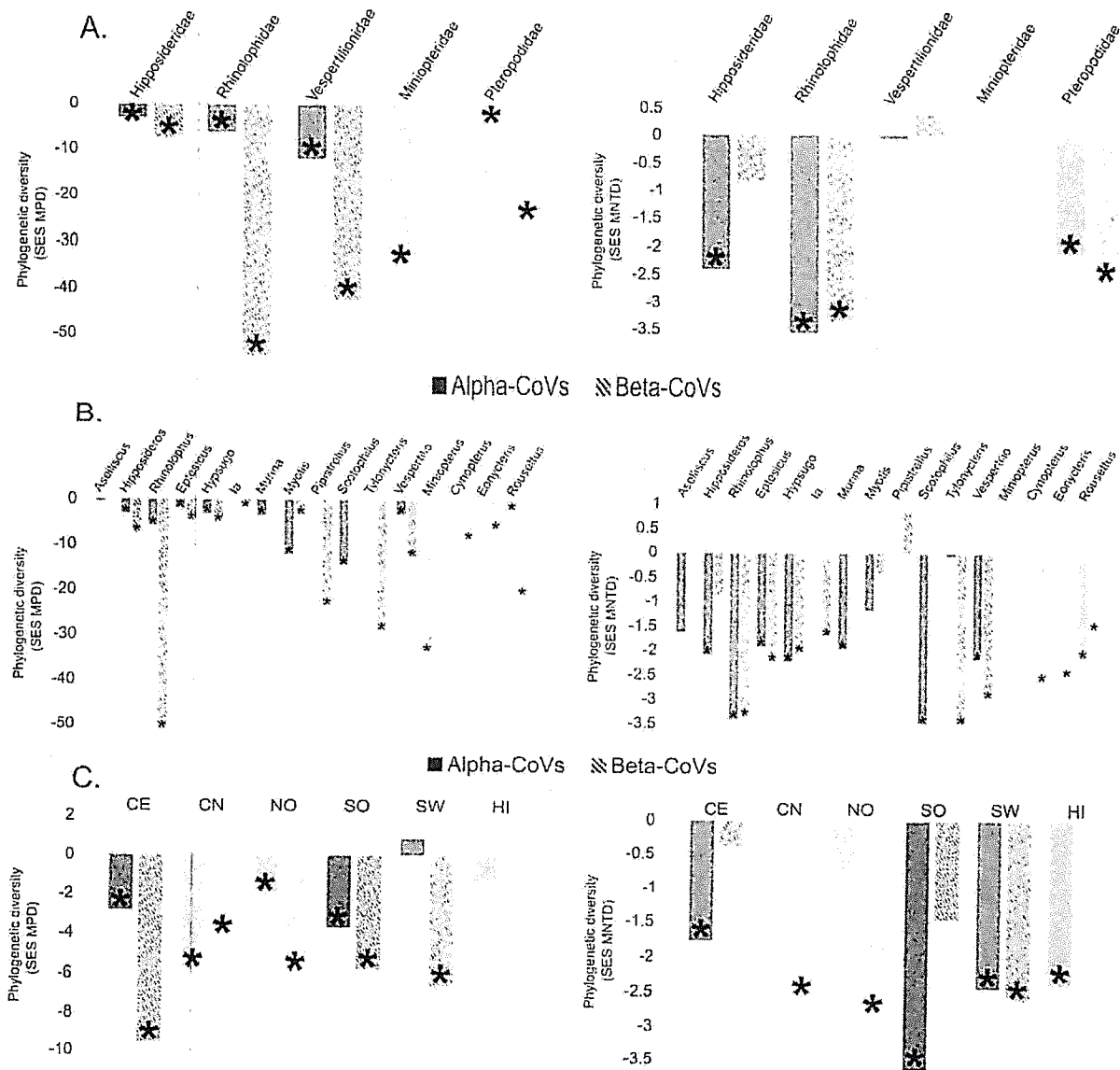


Fig. 9: CoV phylogenetic diversity bat families (A), genera (B), and zoogeographic regions (C): SES MPD, standardized effect size of Mean Phylogenetic Distance (Left); and SES MNTD, standardized effect size of Mean Nearest Taxon Distance (Right). Values departing significantly from null model (p -value < 0.05) indicated with an asterisk. NO, Northern region; CN, Central northern region; SW, South western region; CE, Central region; SO, Southern region; HI, Hainan island.

2.3 Characterization of SADSr-CoV coronaviruses diversity and distributions

In previous project years, our team identified and characterized Swine Acute Diarrheal Syndrome coronavirus (SADS-CoV), a novel swine virus causing outbreaks in farms in multiple Chinese provinces. In this year, we were able to identify SADS-related CoVs in bats from our wild bat sampling. In >17,000 bat and other mammals at 47 sites across southern China, we found 78 new SADSr-CoVs¹¹, all in 9 bat species, with mean prevalence of 0.1 to 37.5%.

Our phylogenetic analysis suggests that pig SADS-CoV recently spilled over from *R. sinicus* or *R. affinis* bats (Fig. 10 Left) However, analysis of full pig viral genomes from 4 initially infected

farms suggests that either the virus evolved as it circulated or that multiple spillover events occurred (Fig. 10 Right).

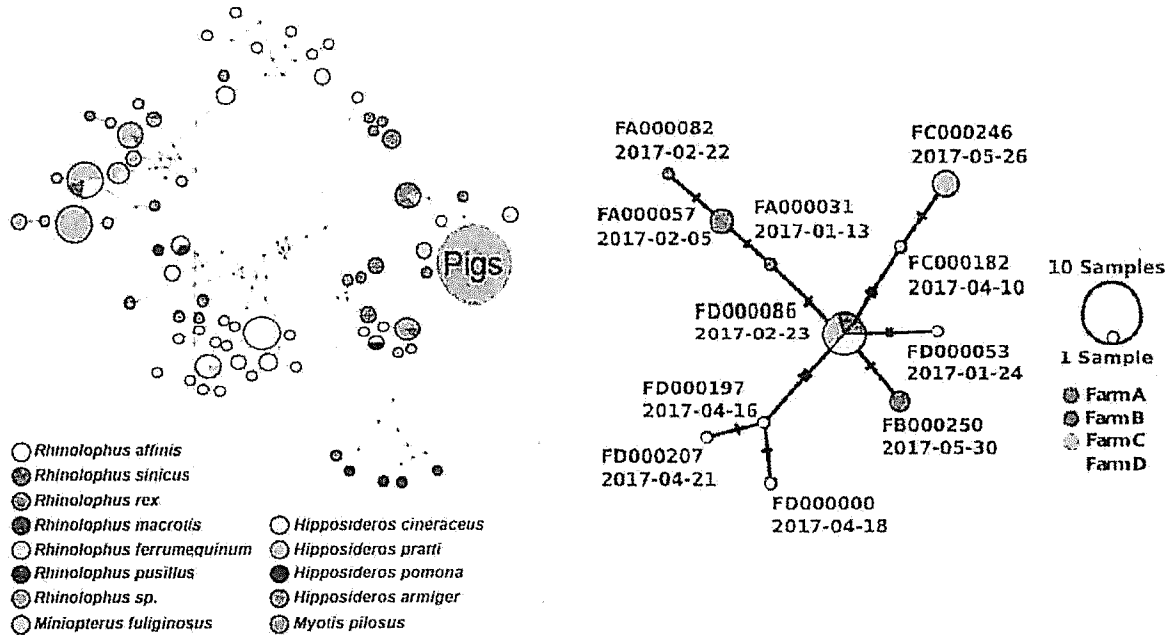


Fig. 10: Left: Median joining network of conserved RdRp gene fragment of 198 unique SADSr-CoV sequences discovered in China under our previous funding. Size of circle proportional to the number specimens with identical viral sequences. Right: Median joining network of SADS-CoV full genome sequence data from 4 infected pigs farms in S. China.

We built species distribution models of the major bat species hosts of SADSr-CoVs across southeast Asia to determine the areas where their ranges intersect with large swine operations similar to those of the original outbreak. We found that these are Southern China (including Taiwan), throughout Vietnam, the Philippines, and Thailand. Compared to other countries,

China had the largest area of bat-pig overlap with 329,847 km² (3.4% of total country area) and 2,127,006 pigs located within predicted bat distributions. By Chinese province, the largest area of overlap was found in Jiangsu (35,226 km² amounting to 34.3% of the province's area and 242,299 pigs within this area). Sichuan had the largest pig population at risk (the pig population within an area that intersects with predicted bat occurrence), at 274,353 heads over 26,015 km² (5.4% of the total area of the province) (Figs. 11 & 12).

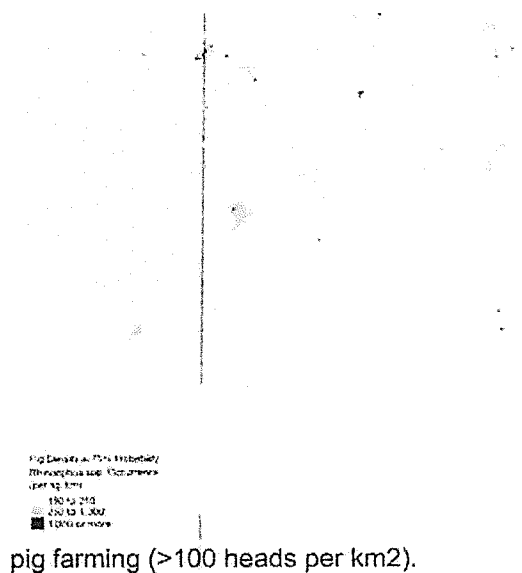


Fig. 5: Areas of bat-pig overlap where probability of SADS-CoV *Rhinolophus* spp. reservoir occurrence is high (>75%) and pig densities are indicative of intensive pig farming (>100 heads per km²).

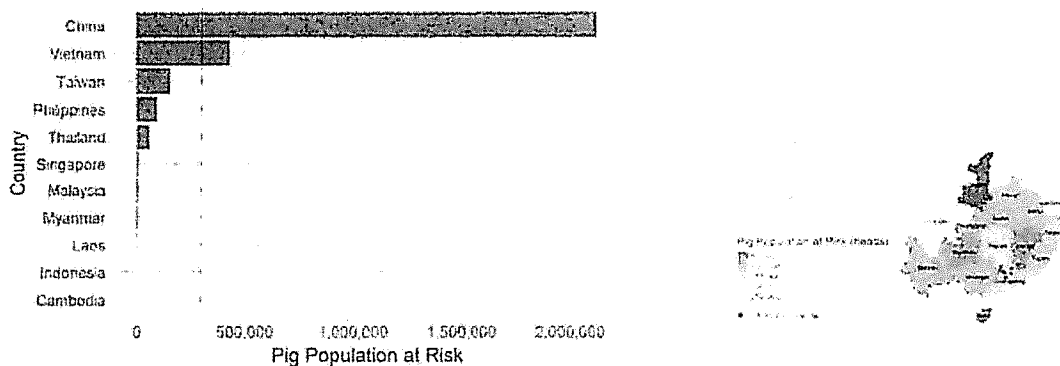


Fig. 6: Top: Country-level, and **Bottom:** province-level estimate of swine populations at-risk based on overlap between modeled populations of bat species known to be SARSr-CoV hosts and large swine operations.

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

3.1 *In vivo* infection of Human ACE2 (hACE2) expressing mice with SARSr-CoV S protein variants

In Year 5, we continued with *in vivo* infection experiments of diverse bat SARSr-CoVs on transgenic mice expressing human ACE2. Mice were infected with 4 strains of SARSr-CoVs with different S protein, including the full-length recombinant virus of SARSr-CoV WIV1 and three chimeric viruses with the backbone of WIV1 and S proteins of SHC014, WIV16 and Rs4231, respectively. Pathogenicity of the 4 SARSr-CoVs was evaluated by recording the survival rate of challenged mice in a 2-week course. All of the 4 SARSr-CoVs caused lethal infection in hACE2 transgenic mice, but the mortality rate vary among 4 groups of infected mice (**Fig. 13a**). 14 days post infection, 5 out of 7 mice infected with WIV1 remained alive (71.4%), while only 2 of 8 mice infected with rWIV1-SHC014 S survived (25%). The survival rate of mice infected with rWIV1-WIV16S and rWIV1-4231S were 50%. Viral replication was confirmed by quantitative PCR in spleen, lung, intestine and brain of infected mice. In brain, rWIV1, rWIV1-WIV16S and rWIV1-4231S cannot be detected 2 days or 4 days post infection. However, rWIV1-SHC014 was detected at all time points and showed an increasing viral titer after infection. The viral load reached more than 10^9 genome copies/g at the dead point (**Fig. 13b**). We also conducted histopathological section examination in infected mice. Tissue lesion and lymphocytes infiltration can be observed in lung, which is more significant in mice infected with rWIV1-SHC014 S (**Fig. 13d**) than those infected with rWIV1 (**Fig. 13c**). These results suggest that the pathogenicity of SHC014 is higher than other tested bat SARSr-CoVs in transgenic mice that express hACE2.

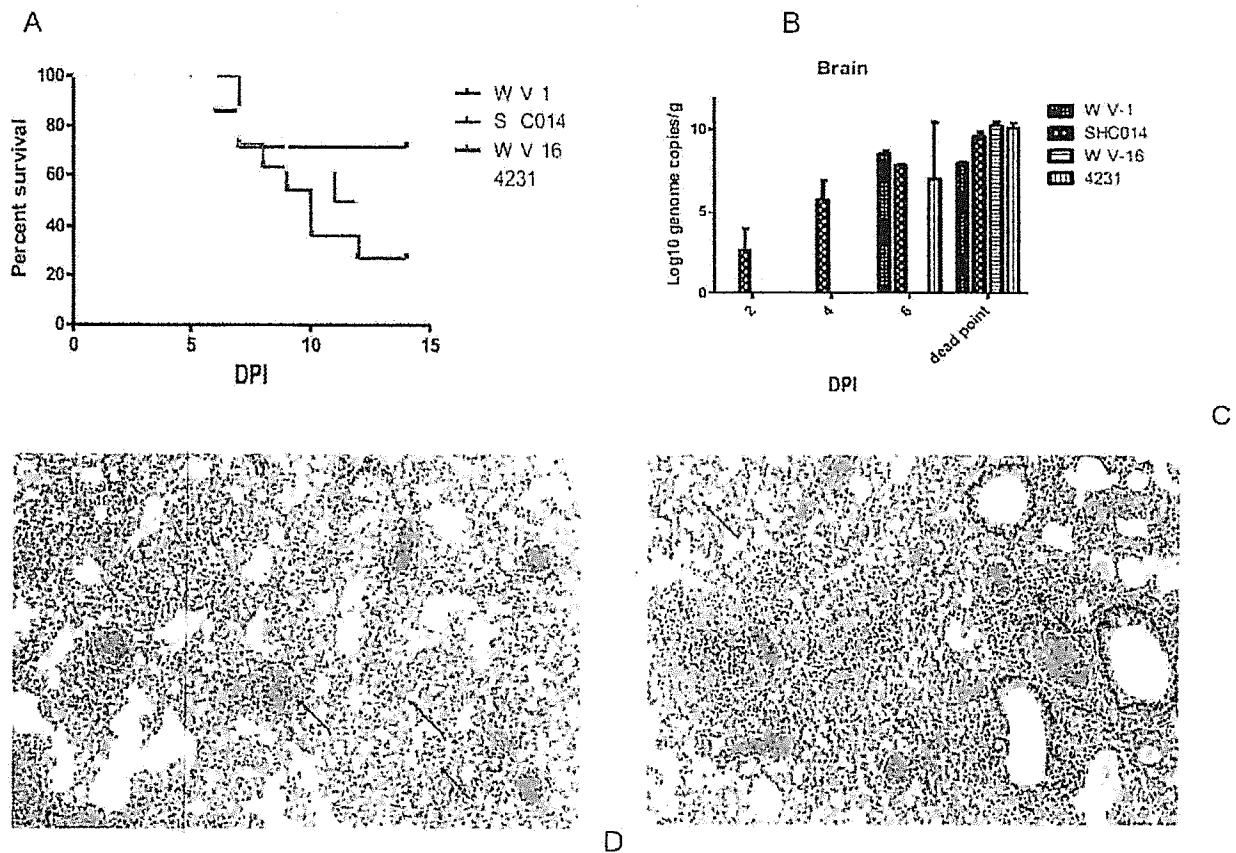


Fig. 13: *In vivo* infection of SARSr-CoV in hACE2-expressing mice. (A) Survival rate of hACE2_mice after infection (B) Viral load in brains of infected hACE2-expressing mice. (C) Histopathological section of lung tissue of mice infected with rWIV1. (D) Histopathological section of lung tissue of mice infected with rWIV1-SHC014 S.

3.2 Assessment of interspecies transmission risk of bat HKU4-related coronaviruses

Taking a similar reverse genetics strategy that we used in SARSr-CoV studies, we constructed the full-length infectious clone of MERS-CoV, and replaced the RBD of MERS-CoV with the RBDs of various strains of HKU4-related coronaviruses previously identified in bats from different provinces in southern China. The full-length MERS-CoV and chimeric viruses with RBDs of HKU4r-CoVs were then rescued. Immunofluorescence assay showed that these chimeric MERS-HKU4rRBD coronaviruses were able to infect human cells from different tissues including lung, liver, intestine and kidney (**Fig. 14 Left**). Moreover, efficient replication of the chimeric HKU4r-CoVs were detected by real-time PCR in HeLa cells that expressed human DPP4 receptor (**Fig. 14 Right**). The results suggest potential risk of the bat HKU4r-CoVs for cross-species infection in humans.

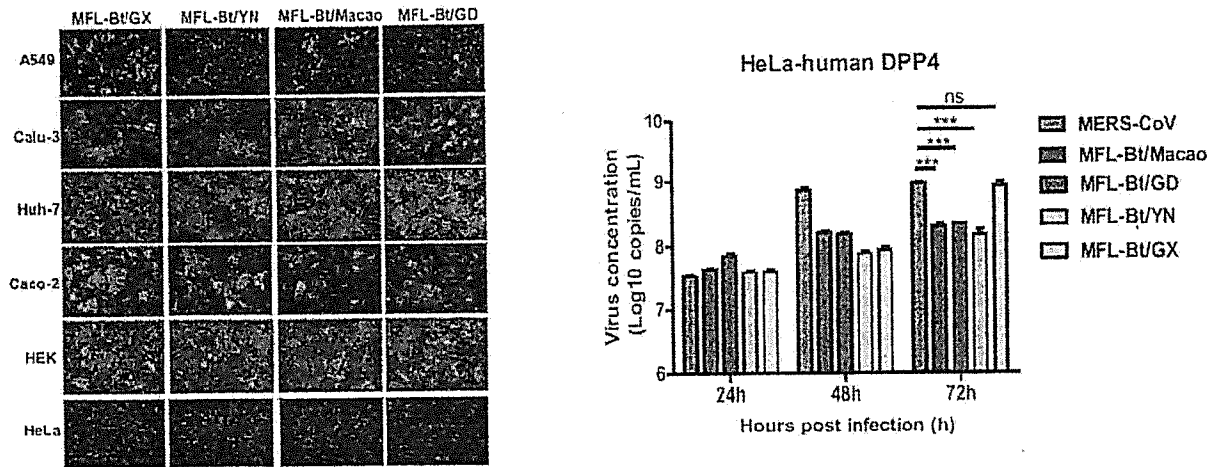


Fig. 7: Left: Immunofluorescence assay confirms Infection of 4 chimeric viruses with the backbone of MERS-CoV and RBD of bat HKU4r-CoVs in different cell lines derived from human tissues. Right: Replication of MERS-HKU4rRBD CoVs in HeLa cells expressing human DPP4 was determined by real-time PCR.

1. Conference and University lectures: We continued to provide 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 provided training to 18 field team members from the Dali College and 4 Wuhan Institute of Virology laboratory team members regarding biosafety and PPE use, bats and rodents sampling.
3. Public outreach: PI Daszak, and Co-investigators Shi, Epstein, and Olival presented the Year 5 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.

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?

No

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
	N	KE, CHANGWEN	PHD	Co-Investigator				Center for Disease Control and Prevention of Guangdong Province	CHINA	NA
	N	ZHANG, YUNZHI	PHD	Co-Investigator				Yunnan Provincial Institute of Endemic Diseases Control & Prevention	CHINA	NA
	N	ZHU, GUANGJIAN	PHD	Co-Investigator				East China Normal University	CHINA	NA
(b) (6)	N	Chmura, Aleksei	BS,PHD	Non-Student Research Assistant						NA
(b) (6)	N	Ross, Noam Martin	PhD	Co-Investigator						NA
(b) (6)	N	Olival, Kevin J.	PHD	Co-Investigator						NA
(b) (6)	N	Zhang, Shu-yi	PHD	Co-Investigator				East China Normal University	CHINA	NA
(b) (6)	N	SHI, ZHENGLI	PhD	Co-Investigator				Wuhan Institute of Virology	CHINA	NA
	N	GE, XINGYI	PHD	Co-Investigator				Wuhan Institute of Virology	CHINA	NA
(b) (6)	N	EPSTEIN, JONATHAN H	MPH,DVM,BA,PHD	Co-Investigator						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

Not Applicable
D.2.b New Senior/Key Personnel Not Applicable
D.2.c Changes in Other Support Not Applicable
D.2.d New Other Significant Contributors Not Applicable
D.2.e Multi-PI (MPI) Leadership Plan Not Applicable

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)?					
<table border="1"><thead><tr><th>Dollar Amount</th><th>Country</th></tr></thead><tbody><tr><td>\$66,500</td><td>CHINA</td></tr></tbody></table>	Dollar Amount	Country	\$66,500	CHINA	
Dollar Amount	Country				
\$66,500	CHINA				

G. SPECIAL REPORTING REQUIREMENTS 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

NOT APPLICABLE

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

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Organization Name: Wuhan Institute of Virology
Country: CHINA

Description of Foreign Component: Principal Laboratory for all Research in China and detailed in our Specific Aims
G.10 ESTIMATED UNOBLIGATED BALANCE Not Applicable
G.11 PROGRAM INCOME Not Applicable
G.12 F&A COSTS Not Applicable

Section 1 - Basic Information (Study 58010)

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:

2.3.a. Inclusion of Individuals Across the Lifespan

2.4. Inclusion of Women and Minorities

2.5. Recruitment and Retention Plan

2.6. Recruitment Status

Not yet recruiting

2.7. Study Timeline

2.8. Enrollment of First Participant (SEE SECTION 6.3)

2.9. Inclusion Enrollment Reports

IER ID#	Enrollment Location Type	Enrollment Location
IER 58010	Foreign	

Inclusion Enrollment Report 58010

1. Inclusion Enrollment Report Title* : China Study Report
2. Using an Existing Dataset or Resource* : Yes No
3. Enrollment Location Type* : Domestic Foreign
4. Enrollment Country(ies): CHN: CHINA
5. Enrollment Location(s):
6. 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 conduct non-exempt human subjects research at more than one domestic site? Yes No N/A

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 this study? Yes No

3.5. Overall structure of the study team

Section 4 - Protocol Synopsis (Study 58010)

4.1. Study Design

4.1.a. Detailed Description

4.1.b. Primary Purpose

4.1.c. Interventions

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

4.1.d. Study Phase

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

4.1.e. Intervention Model

4.1.f. Masking Yes No

Participant Care Provider Investigator Outcomes Assessor

4.1.g. Allocation

4.2. Outcome Measures

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

4.3. Statistical Design and Power

4.4. Subject Participation Duration

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

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

4.6. Is this an applicable clinical trial under FDAAA? (SEE SECTION 6.6)

4.7. Dissemination Plan

I. OUTCOMES

I.1 What were the outcomes of the award?

The aims of our grant (R01AI110964) were to: 1) Analyze the risk that there could be a repeat of the SARS outbreak, due to bat coronaviruses still circulating in China; 2) Work out how we can predict which bat viruses would be most likely to emerge, so that we can prevent new outbreaks; 3) Using lab tests, find out if any of the coronaviruses still present in bat populations in China have the potential to infect people. The overall goal of this work is to help design vaccines and therapeutics against future potentially emerging viruses, work out which communities are on the frontline of a new potential outbreak, and reduce the risk of them being infected by analyzing their risk behavior. During this 5-year period of work, we made significant discoveries leading to 18 peer-reviewed scientific papers, including in some of the world's foremost scientific journals.

Overall, our work shows that bats in China harbor a high number and diversity of coronaviruses, some closely related to SARS-CoV (the virus that caused the SARS pandemic in 2003). We sampled over 16,000 individual bats and found evidence of hundreds of different SARS-related coronavirus genetic sequences. We found out that bats across China harbor these viruses, and that they are common, with 6.7% of bats sampled being positive. Many of these bats are found across China, Southeast Asia, South Asia and beyond, suggesting viruses with zoonotic potential may exist in those regions also. Many of these bats are abundant, and roost and feed close to people and livestock, suggesting high potential for future viral spillover. We also identified one cave system in Yunnan Province with horseshoe bats that had diverse SARSr-CoVs, including some with S proteins able to use human ACE2 as entry receptors. Bats in this cave carried SARSr-CoVs with all unique genetic elements of the SARS-CoV outbreak virus, suggesting that this site may be a potential public health risk.

To analyze which viruses were a potential public health risk, we managed to culture three strains of SARSr-CoVs from bat feces: WIV1, WIV16 and Rs4874. We used the genetic codes of some of the other viruses we found in bats and inserted the spike protein genes of those viruses (the proteins that attach to cells) into the cultured viruses. By doing this experiment we showed that other viruses may also be able to infect human cells, and were able to do this safely without the need to culture large amounts of virus. We also showed that some of these viruses cause SARS-like illness in mice that are adapted to have similar cell surface receptors to people. This work proves that there is a clear and present danger for future emergence of novel SARS-like viruses in people. We also demonstrated that outbreaks can happen in livestock. In 2016-17, we analyzed fecal samples from pigs at 5 farms in South China affected by a fatal diarrheal disease. We discovered a new coronavirus, Swine Acute Diarrheal Syndrome coronavirus (SADS-CoV), and showed that it originates in bats, caused the death of more than 20,000 pigs, but also is able to infect human cells in the lab.

Our work has produced predictive algorithms to map hotspots of viral risk so that public health measures can be taken to protect communities at the frontline of potentially the next SARS pandemic. We have produced new reagents and viral cultures that can be used by labs across the world to design novel vaccines and therapeutics against SARS-CoV and other related viruses that might emerge in the future. Finally, our work has been used directly by the WHO to list SARS-related coronaviruses as one of the highest priority group of pathogens with pandemic potential, so that efforts can be taken to stop a future pandemic before it happens.

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: 04/13/2018
Program Director/Principal Investigator Information: PETER DASZAK , PHD BS Phone number: (b) (6) Email: (b) (6)	Recipient Organization: ECOHEALTH ALLIANCE, INC. ECOHEALTH ALLIANCE, INC. 460 W 34TH ST 17TH FLOOR NEW YORK, NY 100012320 DUNS: 077090066 EIN: 1311726494A1 RECIPIENT ID: NIAID Coronavirus
Change of Contact PD/PI: N/A	
Administrative Official: ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 Phone number: (b) (6) Email: (b) (6)	Signing Official: ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 Phone number: (b) (6) Email: (b) (6)
Human Subjects: Yes HS Exempt: No Exemption Number: Phase III Clinical Trial:	Vertebrate Animals: Yes
hESC: No	Inventions/Patents: No

B. ACCOMPLISHMENTS

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

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then hundreds of novel bat-CoVs have been discovered (including >260 by our group). These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a largescale human-wildlife interface, and high risk of future emergence of novel CoVs.

To understand the risk of zoonotic CoV emergence, we propose to examine 1) the transmission dynamics of bat-CoVs across the human-wildlife interface, and 2) how this process is affected by CoV evolutionary potential, and how it might force CoV evolution. We will assess the nature and frequency of contact among animals and people in two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In the markets we hypothesize that viral emergence may be accelerated by heightened mixing of host species leading to viral evolution, and high potential for contact with humans. In this study, we propose three specific aims and will screen free ranging and captive bats in China for known and novel coronaviruses; screen people who have high occupational exposure to bats and other wildlife; and examine the genetics and receptor binding properties of novel bat-CoVs we have already identified and those we will discover. We will then use ecological and evolutionary analyses and predictive mathematical models to examine the risk of future bat-CoV spillover to humans. This work will follow 3 specific aims:

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

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

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

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

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: 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 hACE2-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 Agricultural 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.

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**).

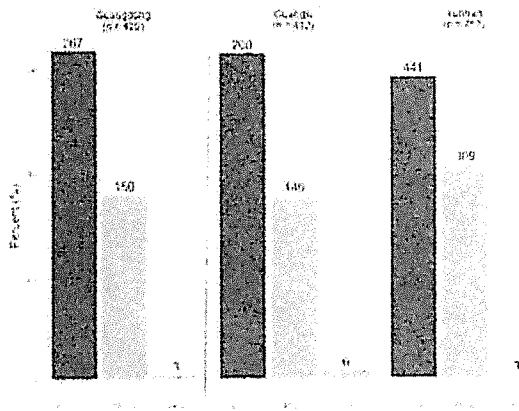


Figure 1: Gender of respondents

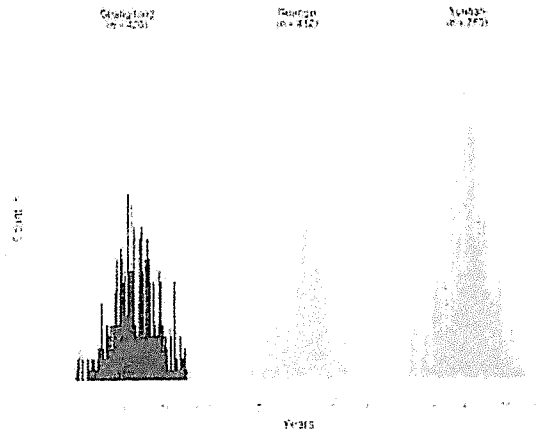


Figure 2: Age distribution of respondents.

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%).

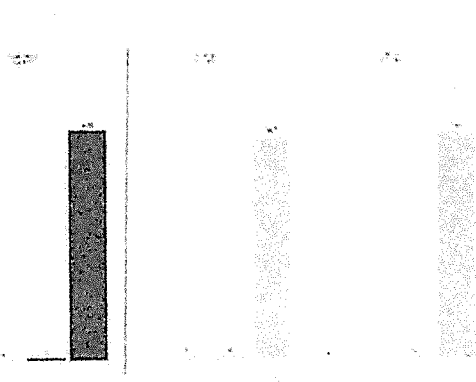


Figure 3: Duration of residency.

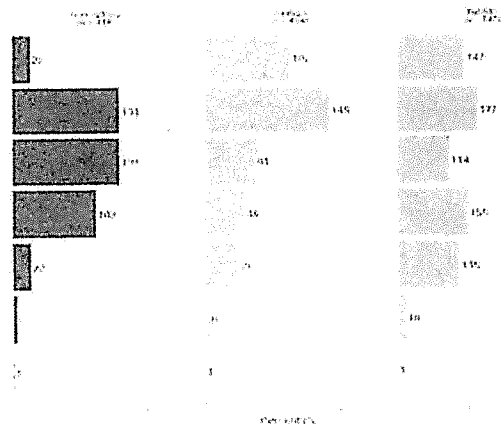


Figure 4: Family annual per capita income (RMB).

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.

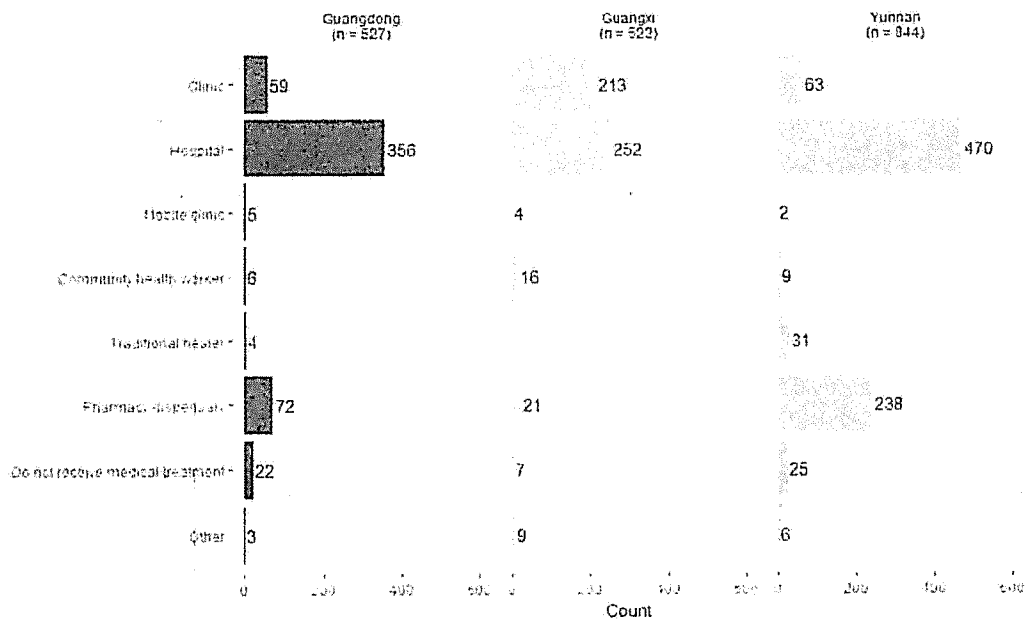
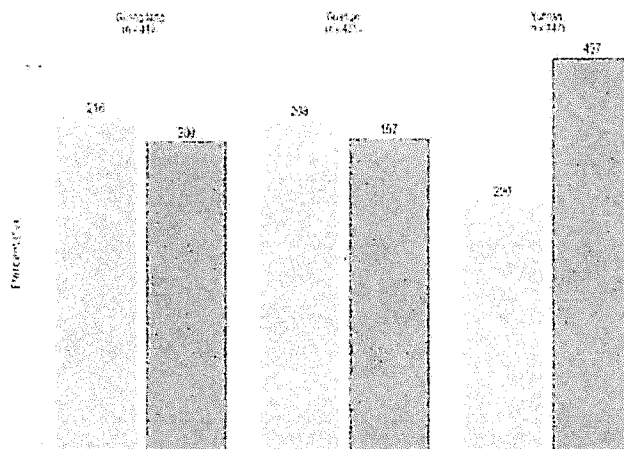


Figure 7: Location where care was usually received for illness or infection.

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.

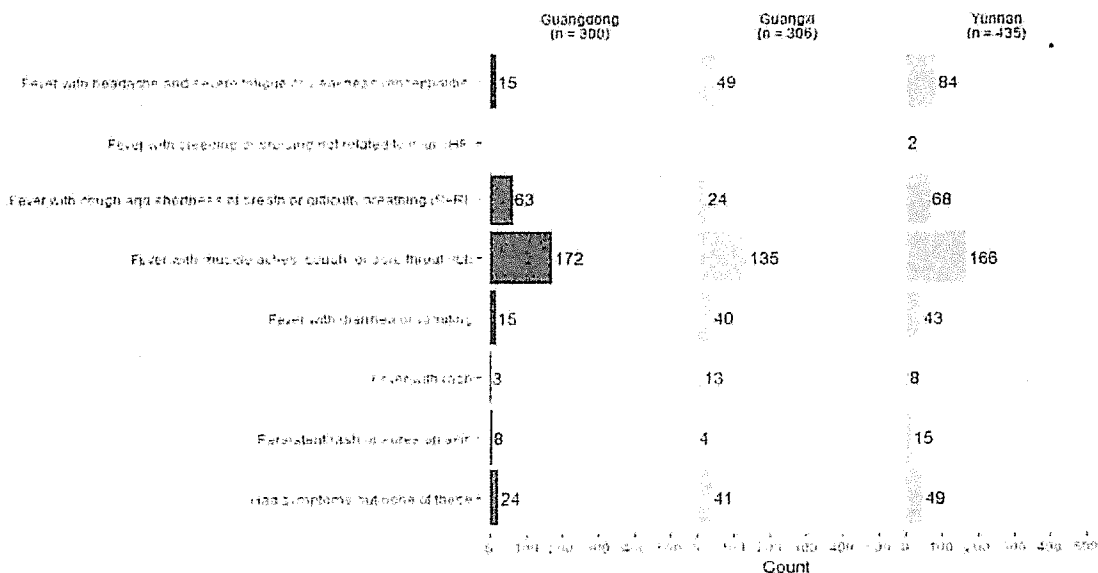
The proportion of respondents who had an unusual illness with any of the above-mentioned symptoms in their lifetime varied slightly by province. Between the three provinces, Yunnan had the fewest number of respondents who reported experiencing the symptoms provided (38.8%), compared to Guangdong and 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**).

Figure 8: Respondent's experience of unusual illnesses.



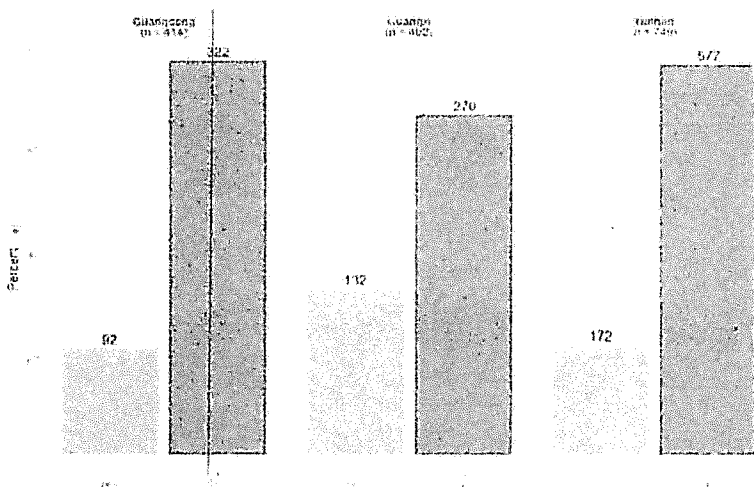
Across all three provinces, among those who had experienced any symptoms of unusual illness in their lifetimes, those associated with ILI were the most commonly reported. In Guangdong province, this was followed by symptoms associated with SARI, then by other symptoms not mentioned in the survey. In 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).

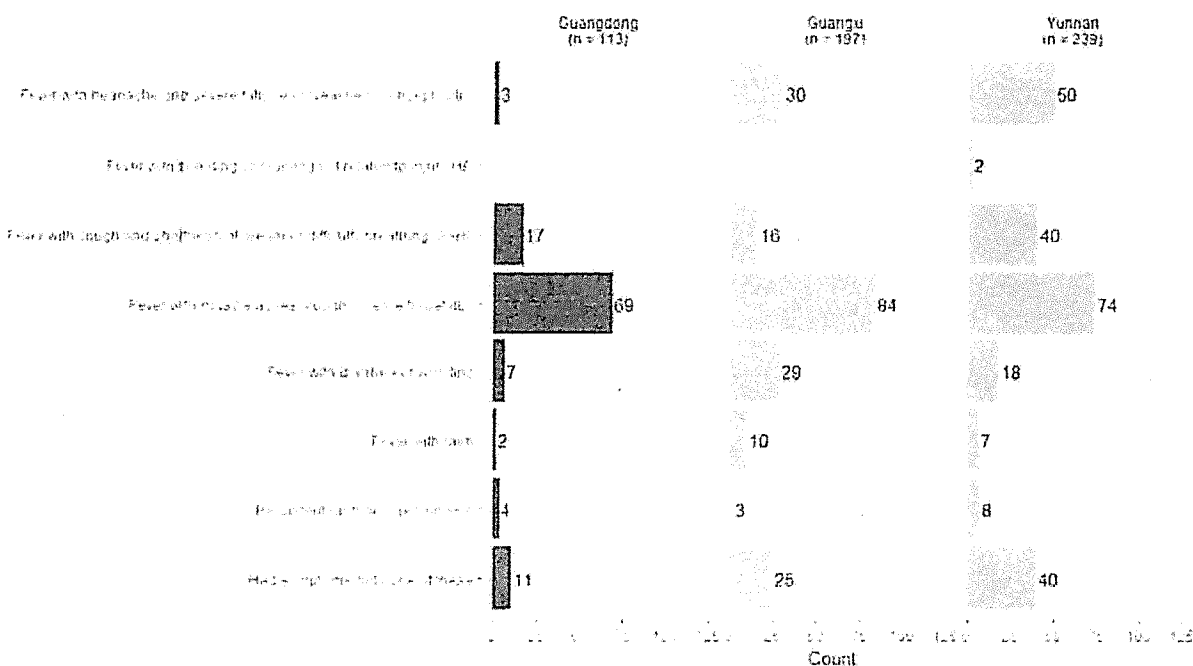
Figure 10: Whether respondents had experienced symptoms associated with an unusual illness, in the past year.



Of the respondents who reported having symptoms of unusual illness in the past year, across all three provinces, symptoms associated with 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).

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

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.

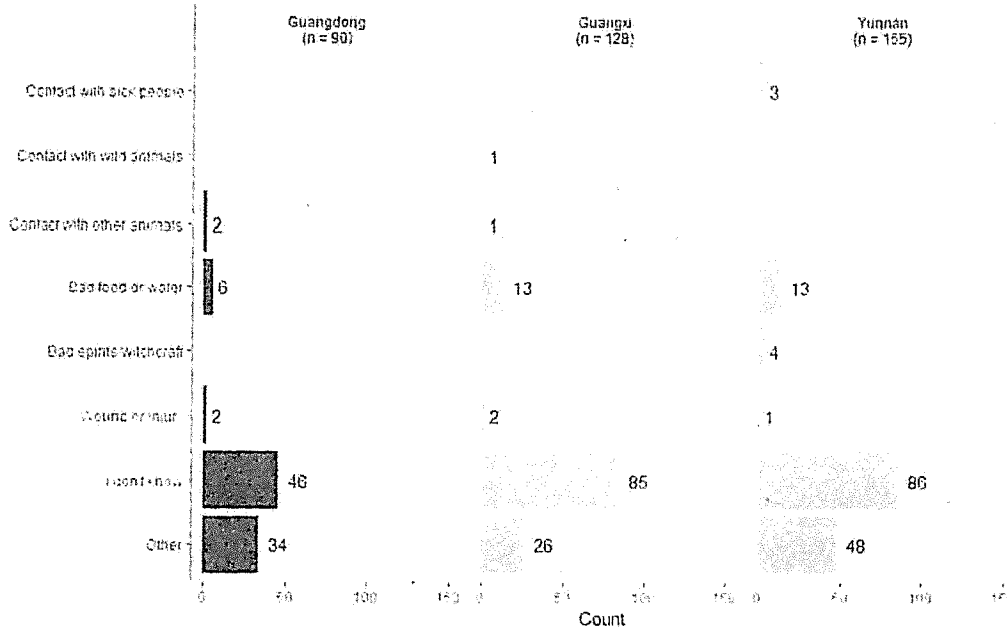
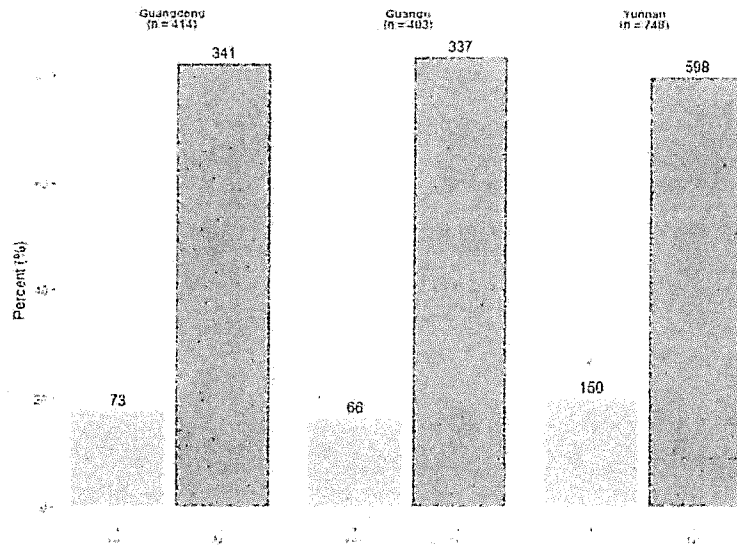


Figure 12: Reported cause of sickness in the past year.

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



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).

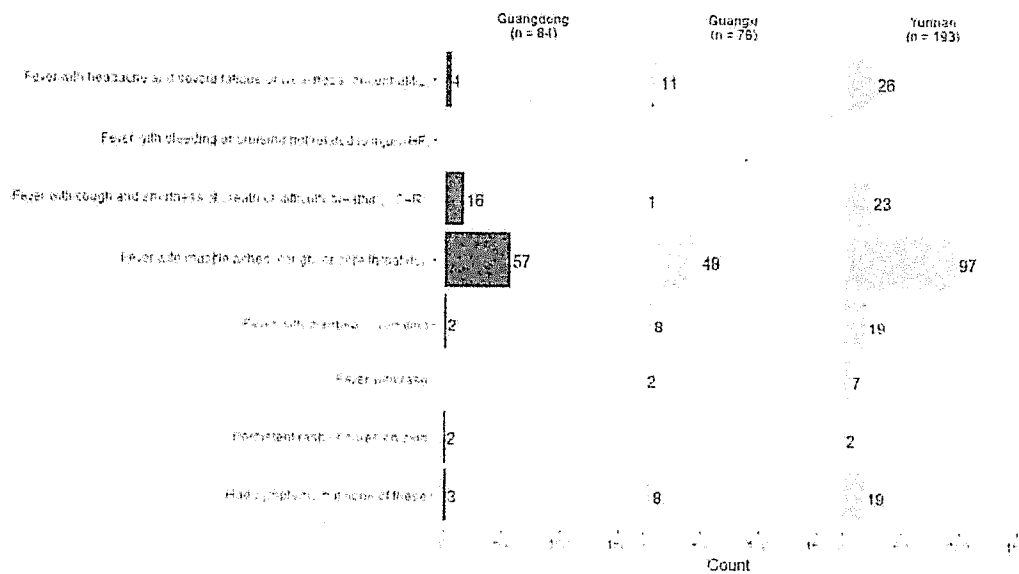


Figure 14: Symptoms of household members who were ill, in past year.

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).

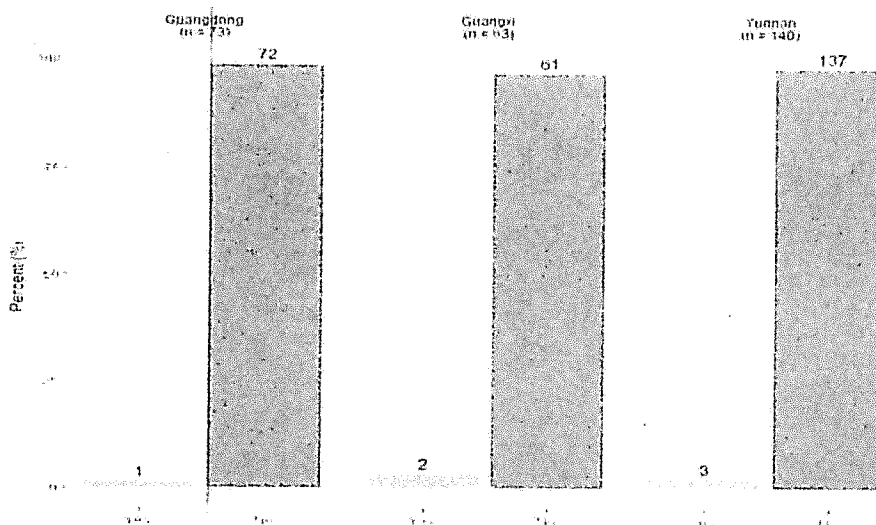
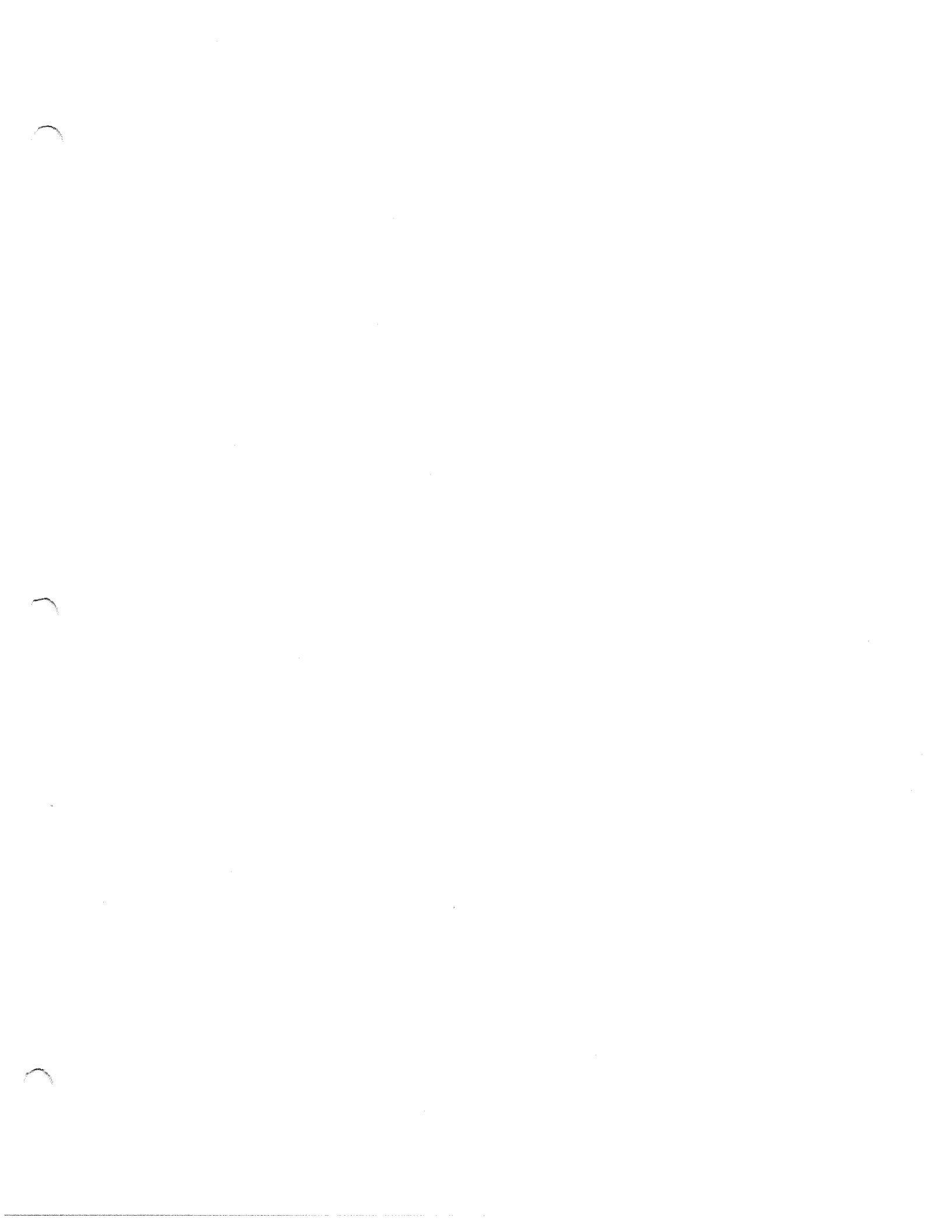


Figure 15: Whether household members died from illness, in the past year.





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, within a respondent's lifetime.

Respondents who reported having animal contact in their lifetime were also asked to indicate if they had the same type of animal contact in the past year (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).

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).

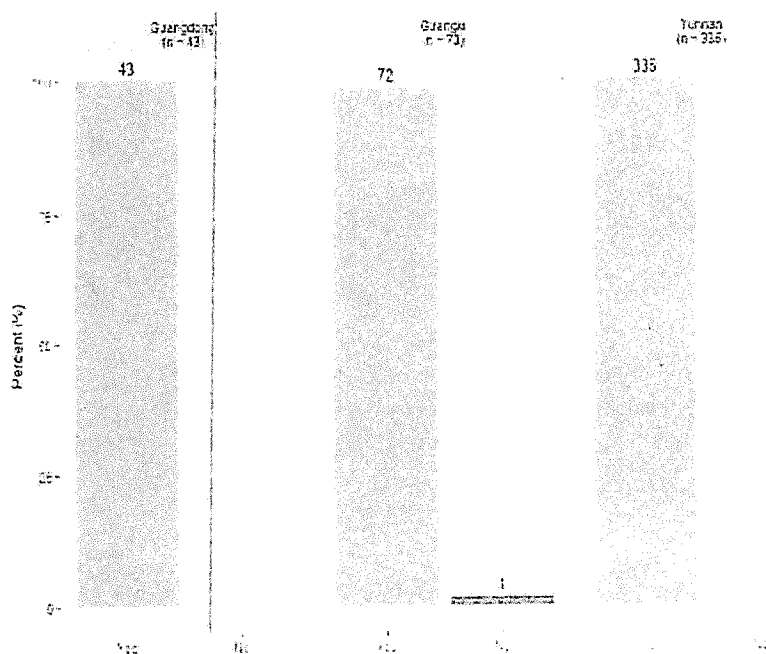
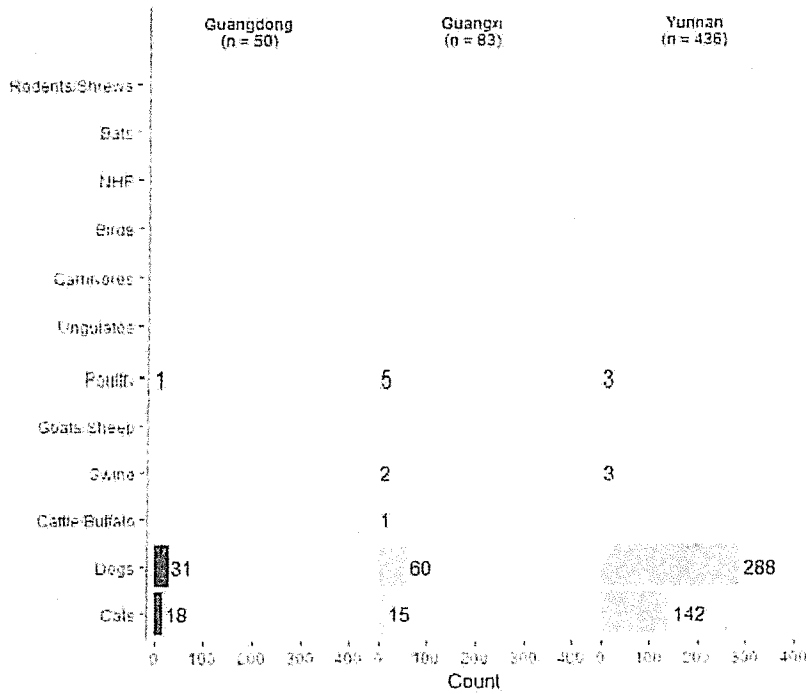


Figure 16a (top) & b (below): (a) Whether respondents had lived with an animal as a pet, in the past year, and (b) among those who had, types of animal kept as pets.



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).

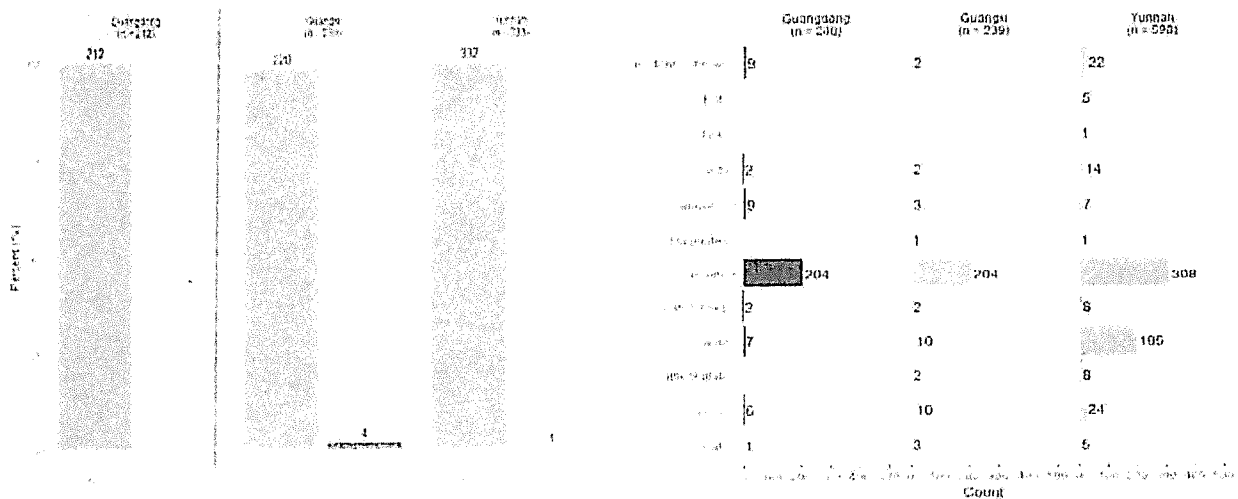
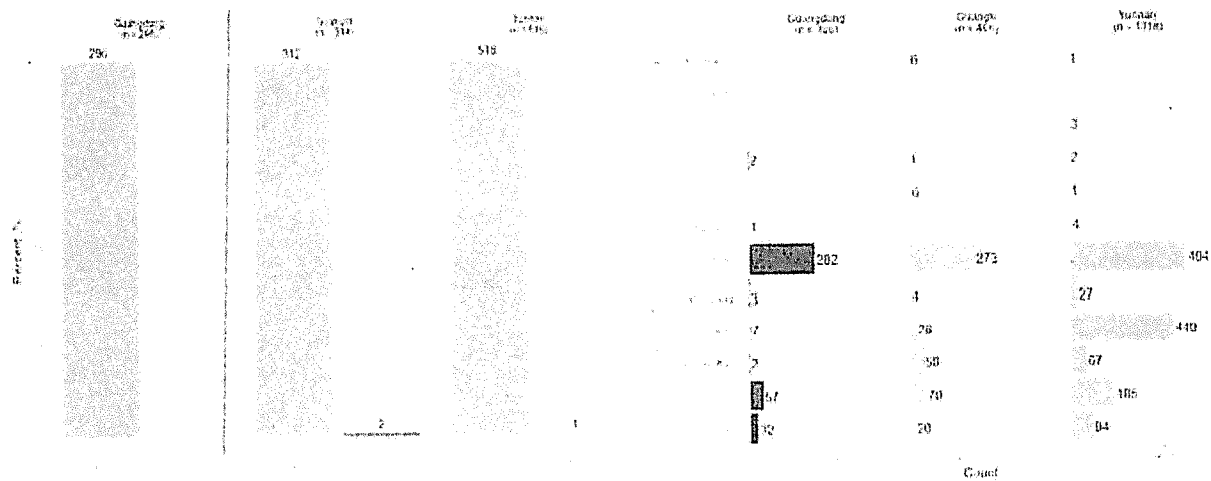


Figure 17a & b: (a) Whether respondents had handled live animals, in the past year, and (b) among those who had, types of live animals handled.

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).

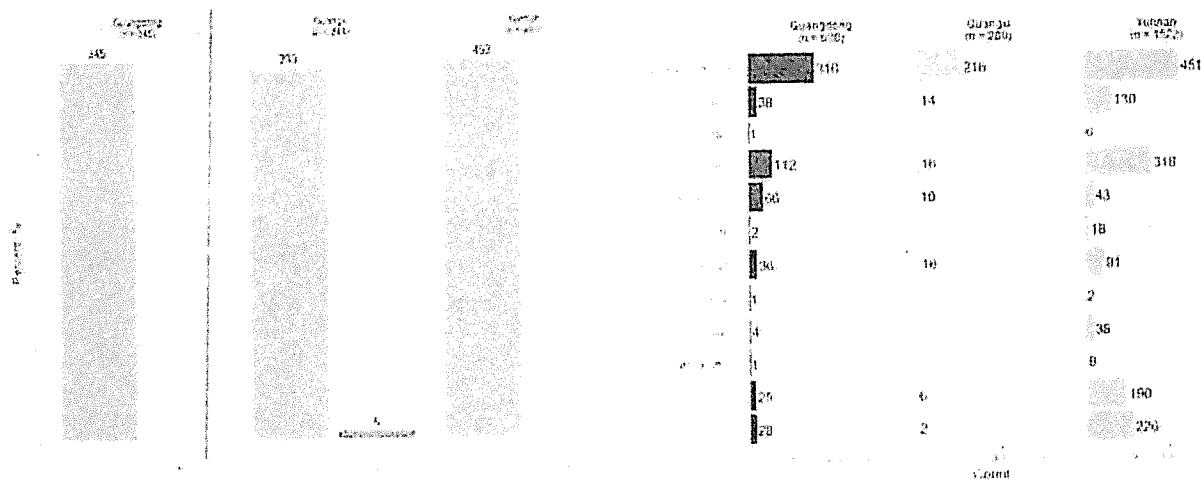


Figure 19a & b: (a) Whether respondents had animals come inside dwelling, in the past year, and (b) among those who had, types of animals in dwelling.

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).

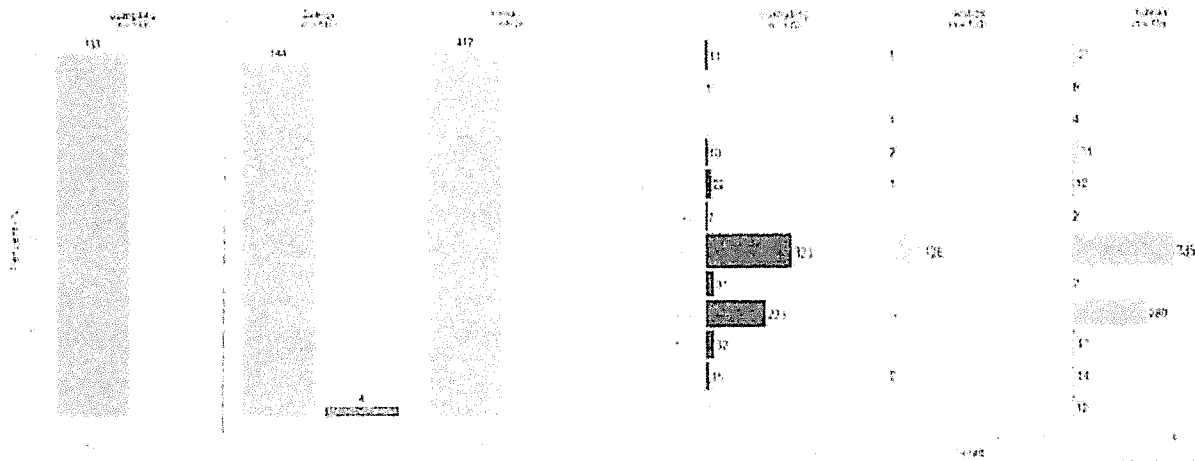


Figure 20a & b: (a) Whether respondents had cooked or handled meat, organs or blood from a recently killed animal, in the past year, and (b) among those who had, types of animals whose meat, organs or blood was cooked or handled.

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.

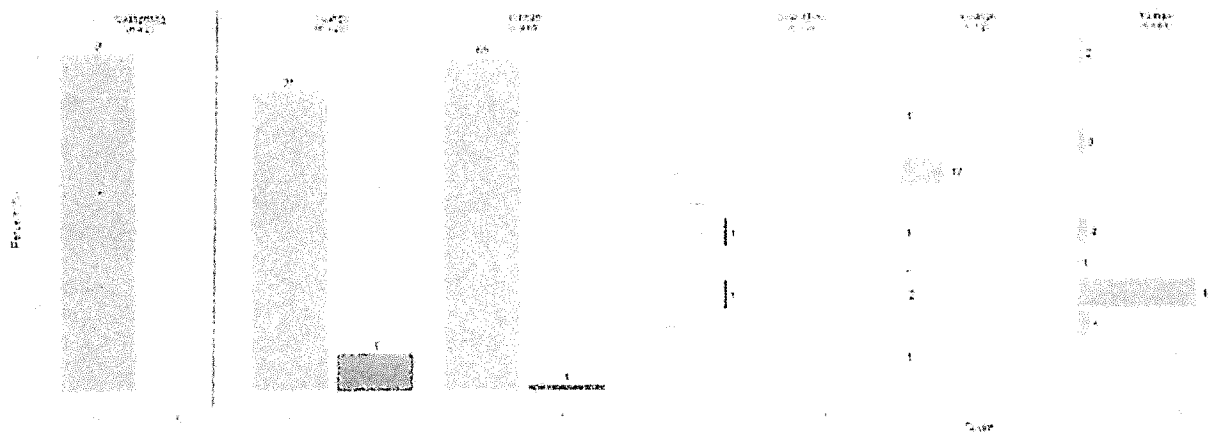


Figure 21 a & b: (a) Whether respondents had eaten raw or undercooked meat or organs or blood, in the past year, and (b) among those who had, types of animals whose meat, organs or blood were eaten raw or undercooked.

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 Guangdong, 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**).

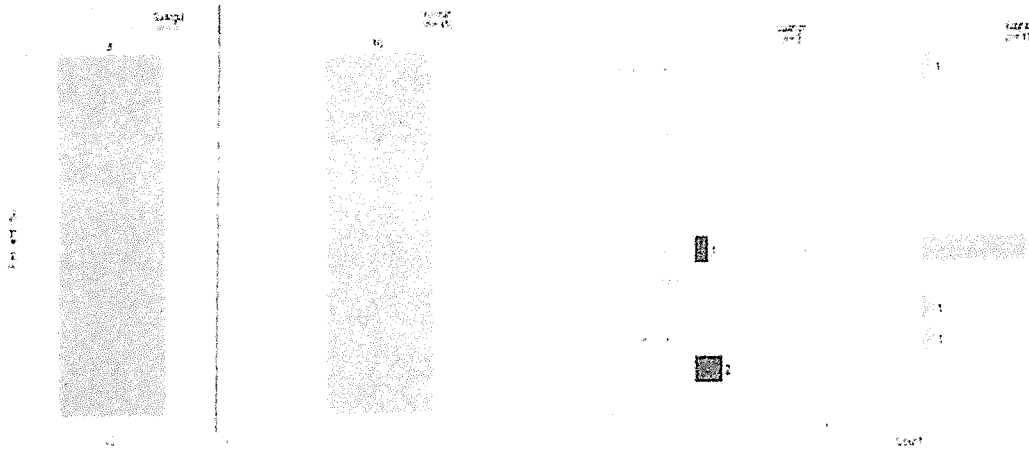


Figure 22 a & b: (a) Whether respondents had found a dead animal and collected it to eat, share, or sell, in the past year, and (b) among those who had, types of animals that were found dead and collected to eat, share, or sell.

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**).

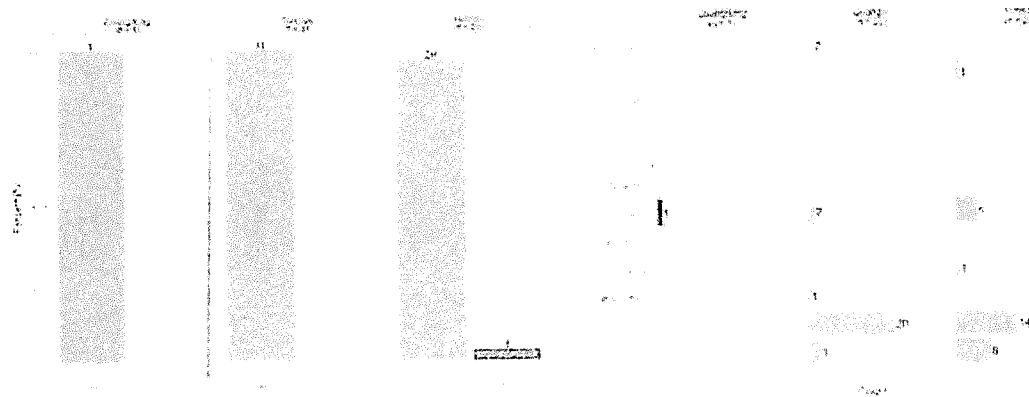


Figure 23 a & b: (a) Whether respondents had been scratched or bitten by an animal, in the past year, and (b) among those who had, types of animals that scratched or bit respondents.

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**).

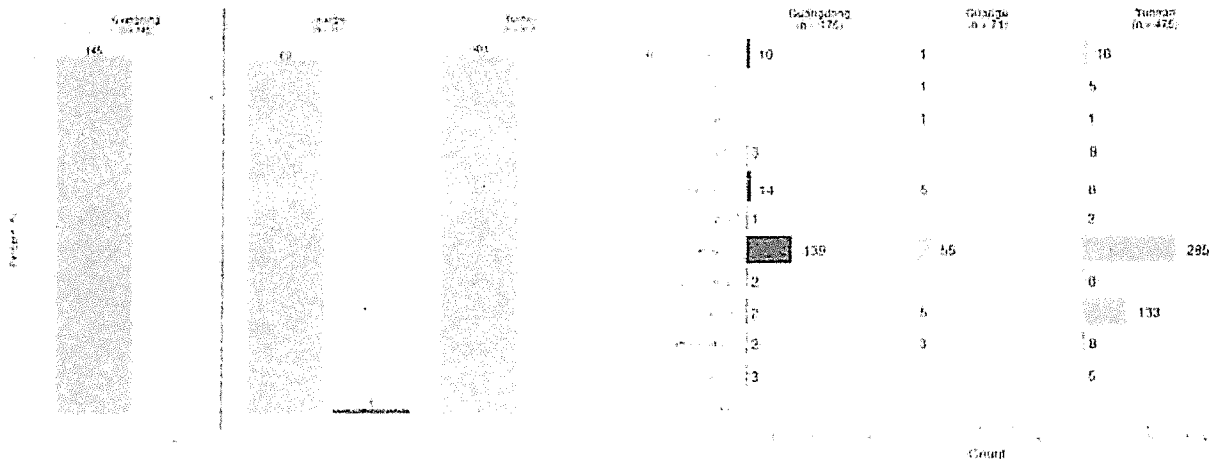


Figure 24 a & b: (a) Whether respondents had slaughtered an animal, in the past year, and (b) among those who had, types of animals slaughtered.

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**).

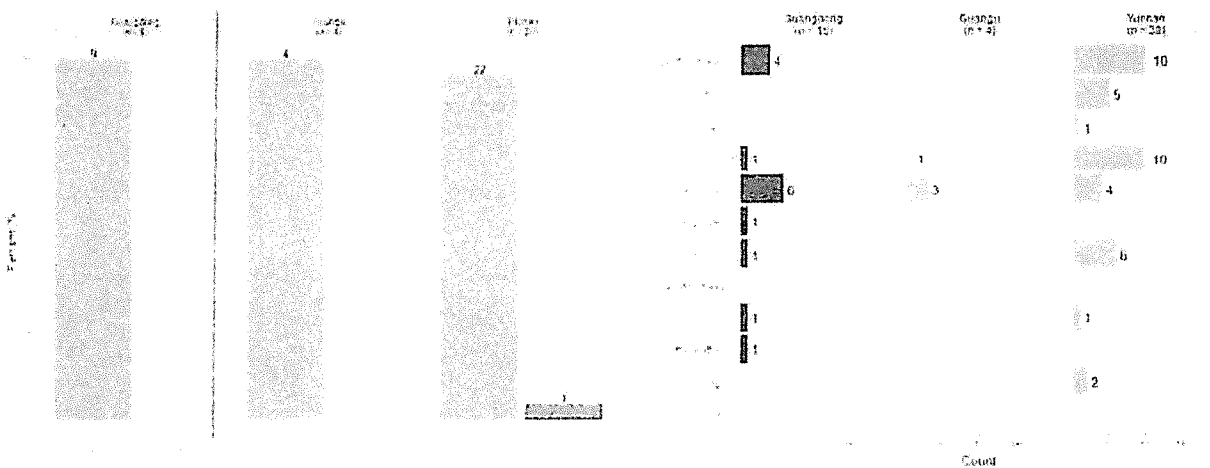


Figure 25 a & b: (a) Whether respondents had hunted or trapped an animal, in the past year, and (b) among those who had, types of animals hunted or trapped.

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).

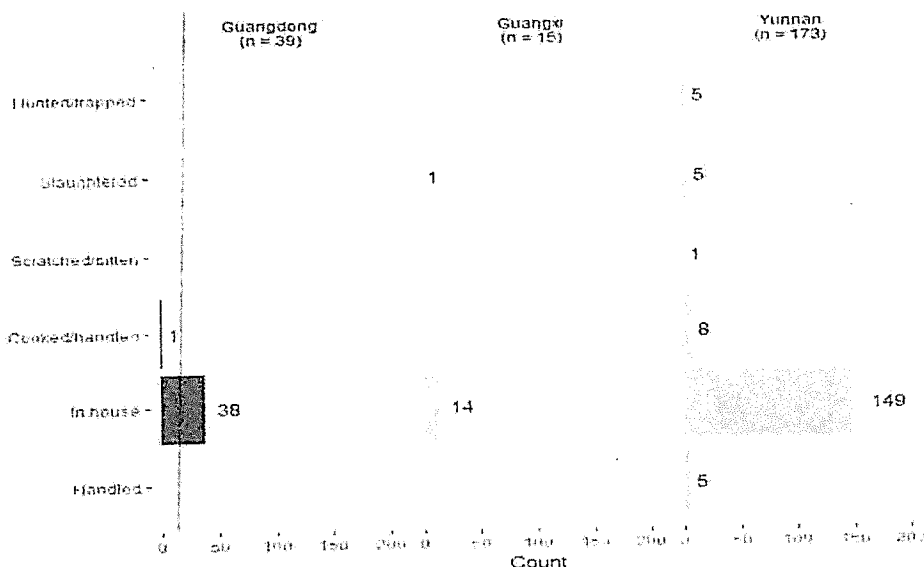


Figure 26: Types of bat contact.

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).

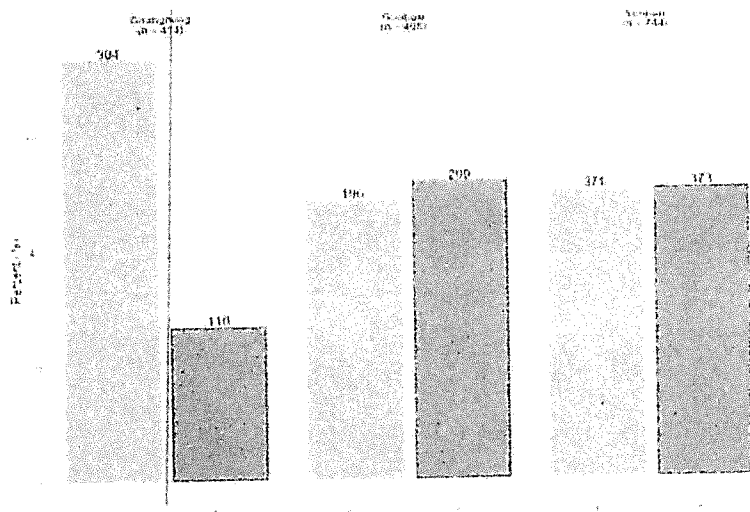


Figure 27: Whether respondents thought that animals can spread disease.

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**)

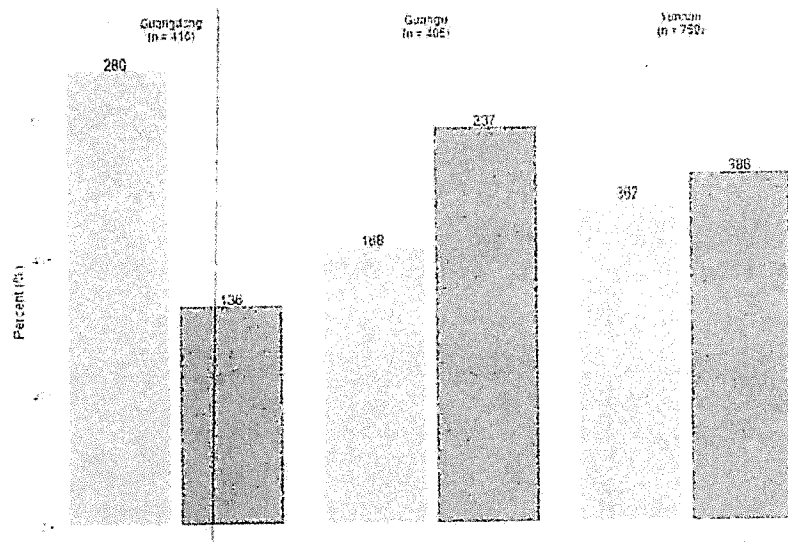


Figure 28: Whether respondents were worried about diseases or disease outbreaks in animals at wet markets.

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**).

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).*

Links Between ELISA Results and Behavior

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

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 than 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 10 th 2017	Hezhou, Guangxi	6	6
May 11-12 th 2017	Chongzuo, Guangxi	67	67
May 13 th 2017	Nanning, Guangxi	66	66
May 17 th , 2017	Beihai, Guangxi	23	23
May 19 th 2017	Chongzuo, Guangxi	36	36
May 21 st 2017	Yangshan, Qingyuan, Guangdong	46	46
May 22 nd , June 7 ^h 2017	Huidong, Huizhou, Guangdong	103	103
June 9 th 2017	Nanning, Guangxi	71	71
June 9 th 2017	Ningming, Chongzuo, Guangxi	63	63
September 10 th 2017	Huidong, Huizhou, Guangdong	100	100
September 11 th 2017	Yingde, Guangdong	90	90
Total		671	671

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

Species	Guangdong	Guangxi	Total
<i>Rhinolophus sinicus</i>	9/27	6	9/33
<i>Rhinolophus rex</i>		4	4
<i>Rhinolophus pusilus</i>	1	2	3
<i>Rhinolophus pearsoni</i>	5		5
<i>Hipposideros armiger</i>	24	8	32
<i>Hipposideros larvatus</i>	9	9	18
<i>Hipposideros pomona</i>		20	20
<i>Hipposideros pratti</i>	26		26
<i>Aselliscus stoliczkanus</i>		1	1
<i>Miniopterus fuliginosus</i>	1		1
<i>Miniopterus pusillus</i>	29/39		29/39
<i>Myotis chinensis</i>	2/27		2/27
<i>Myotis daubentonii</i>	2		2
<i>Myotis ricketti</i>	86/178		86/178
<i>Pipistrellus abramus</i>		2	2
<i>Pipistrellus pipistrellus</i>		2	2
<i>Scotophilus kuhli</i>		24/137	24/137
<i>Tylonycteris pachypus</i>		4/115	4/115
<i>Tylonycteris robustula</i>		3	3
<i>Cynopterus sphinx</i>		23	23
Total	121/339	236/82	154/671

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

A high prevalence of HKU6-related coronaviruses (48.3%), *Scotophilus coronavirus* 512 (17.5%), and coronavirus 1B (71.8%) was detected in *Myotis ricketii*, *Scotophilus 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).

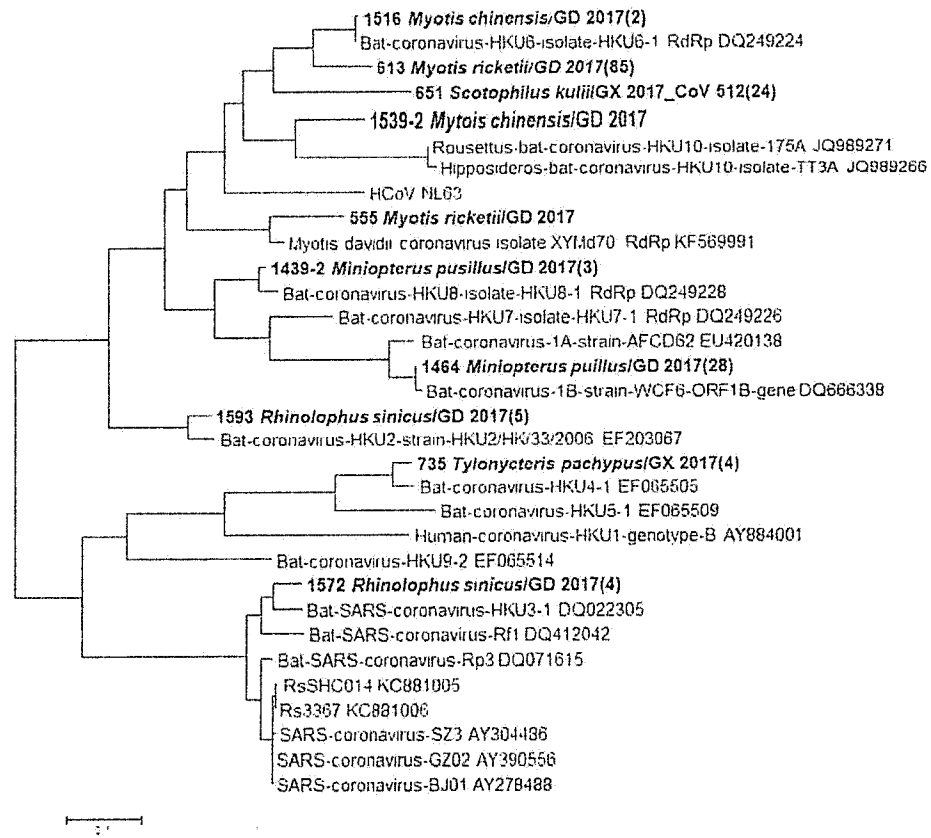


Figure 29: Phylogenetic analysis of partial RdRp gene of CoV (440-nt partial sequence)

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*, *Roussettus leschenaultia* and an unclassified *Roussettus* species. These viruses are classified into two betacoronavirus species, HKU9-CoV and GCCDC1-CoV. All HKU9-related viruses (n=46) were found in *Roussettus* 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

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-genital routes.

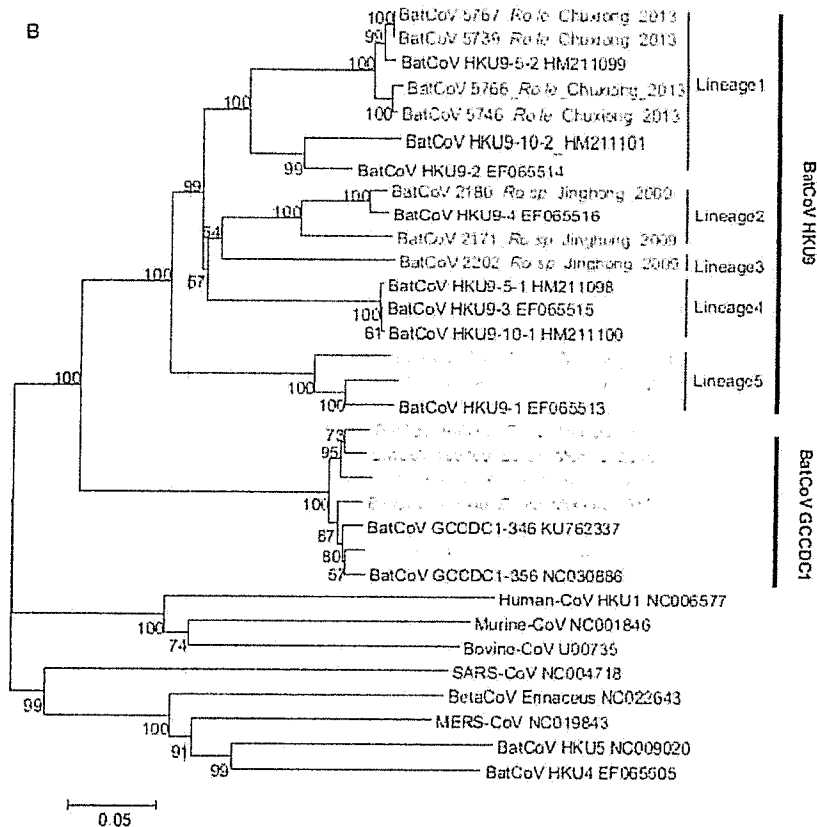


Figure 30. Phylogenetic analysis of full-length N gene of HKU9 and GCCDC1 CoVs

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).

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

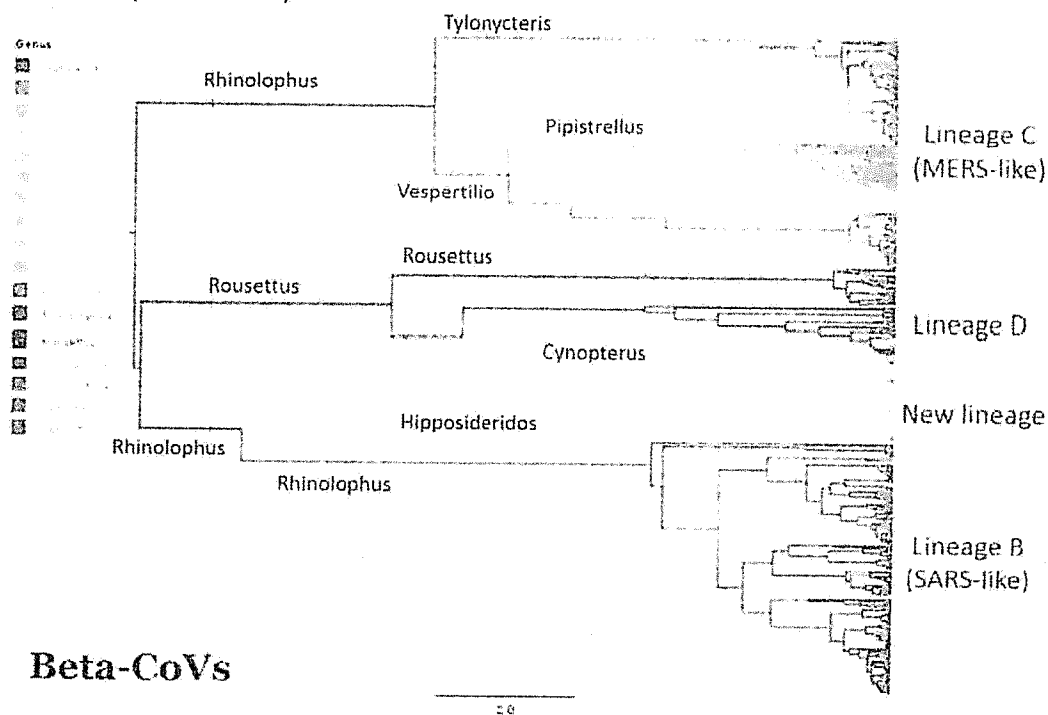


Figure 31. Ancestral host reconstruction for Beta-CoVs, at a host genus level.

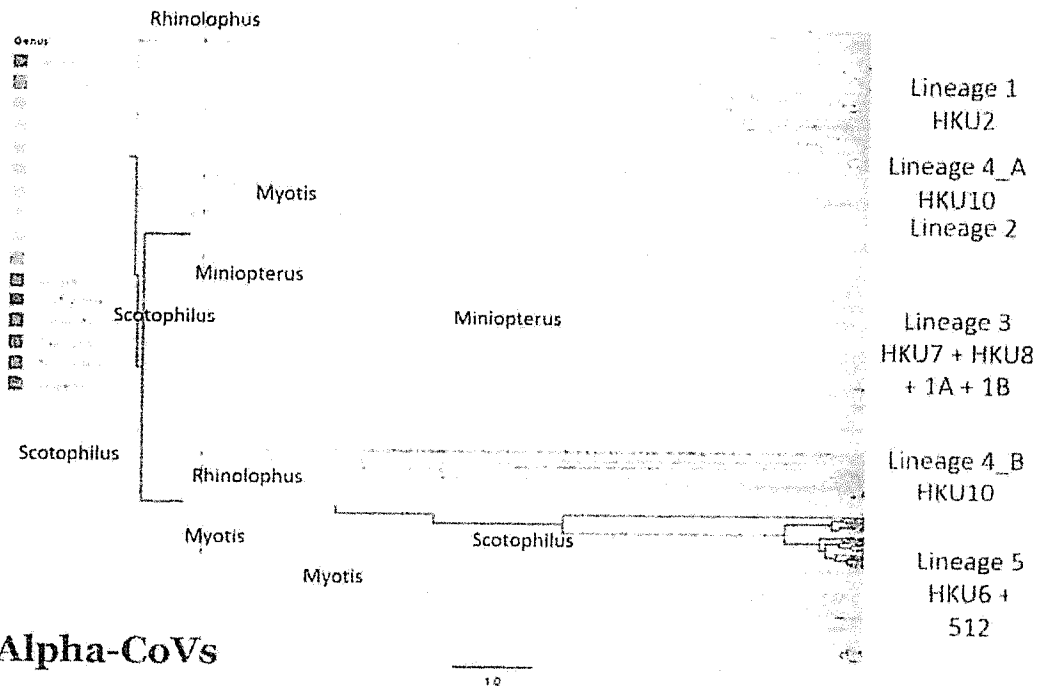


Figure 32. Ancestral host reconstruction for Alpha-CoVs, at a host genus level.

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.

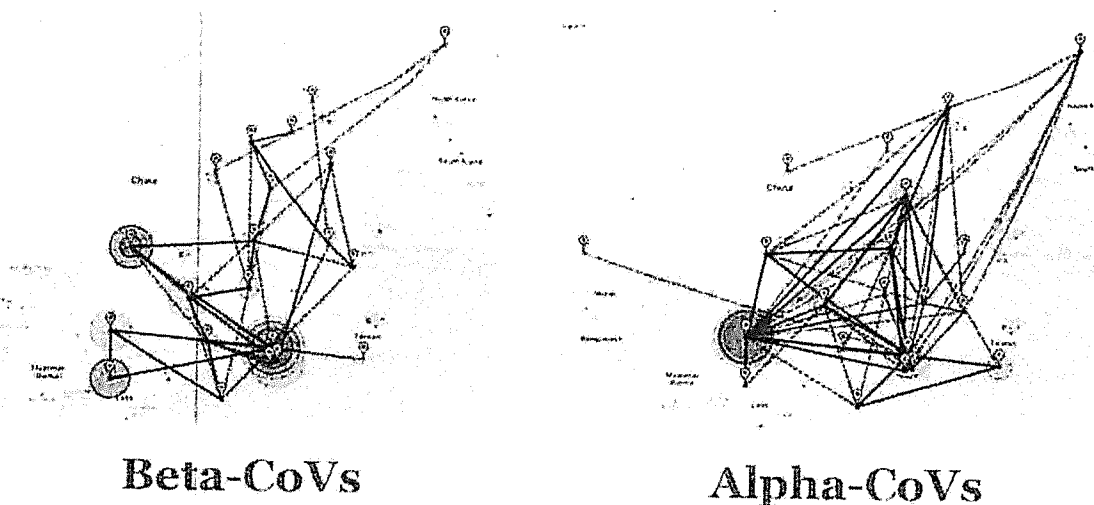


Figure 33. Ancestral location reconstruction for Beta- and Alpha-CoVs. The bigger the circle is, the more ancestral the corresponding node is.

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.

A

	467								
MERS	ENYKQSFANP	TCLILATVPH	NLTT---ITK	PLKYSYINKC	SRLLSDD-RT	515			
422	YNYKQSFANP	TCRIFATAPA	NLT----ITK	PSSYSFISKC	SRLTGDNHSI	516			
845	FNYKQSFANP	TCRIFATAPA	NLT----ISK	PSSYSYISKC	SRLTGDNQHI	517			
HKU4	YNYKQSFANP	TCRVMSVLA	NVT----ITK	PHAYGYISKC	SRLTGANQDV	517			
SC2013	ENYKQDFSNP	TCRILATVPA	NLSASGLLPK	PSNYVWLSEC	YQNSFTG---	488			
Neo	FNYNQDYSNP	SCRIHSKVNS	SIG----ISY	AGAYSYITNC	NYGATNK---	512			
PDF-2180	FNYNQDYSNP	SCRIHSKVNS	SVG----ISY	SGLYSYITNC	NYGGFNK---	513			
HKU5	ENYKQDFSNP	TCRVLATVPQ	NLTT---ITK	PSNYAYITEC	YKTSAYG---	518			
	:*.*	*: : . . .	: :	: . .	* : : : *				
	513								
MERS	EVPQLVNAQ	YSFCVSIVPS	TWEDGDYR	KQLSPLLEGGG	WLVASGSTVA	562			
422	ETPIVINPGE	YSICKNFAPN	GFSQDGDYFT	RQLSQLEGGG	ILVGVGSVTP	566			
845	ETPITINPGE	YSICRGFAPN	GLSEDDQVFT	RQLSDYEGGG	TLVGVGNTVP	567			
HKU4	ETPLYINPGE	YSICRDFSPG	GFSQDQVFK	RTLTPQEGGG	LLIGVGTVP	567			
SC2013	KNFQYVKAGQ	YTPCLGLAAN	GFEKSYQTHR	DPV-----S	KLAVTGVVTP	532			
Neo	DDVVKEGGRA	SQQCITGALN	S-PTTGLWA	YNF-----GG	VFYRVSRLTY	556			
PDF-2180	DDVVKEGGRA	SQPCVTGALN	S-PTNGQVWS	FNF-----GG	VFYRVSRLTY	557			
HKU5	KNYLYNAPGA	YTPCLSLASR	GFSKYQSHS	D-----G	ELTTTCYIYP	561			

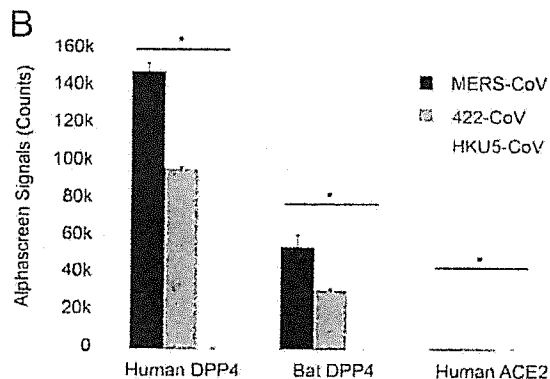


Figure 34. *BtCoV/II/GD/2014-422 RBD analysis (a) and DPP4-binding assay (b)*

***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.

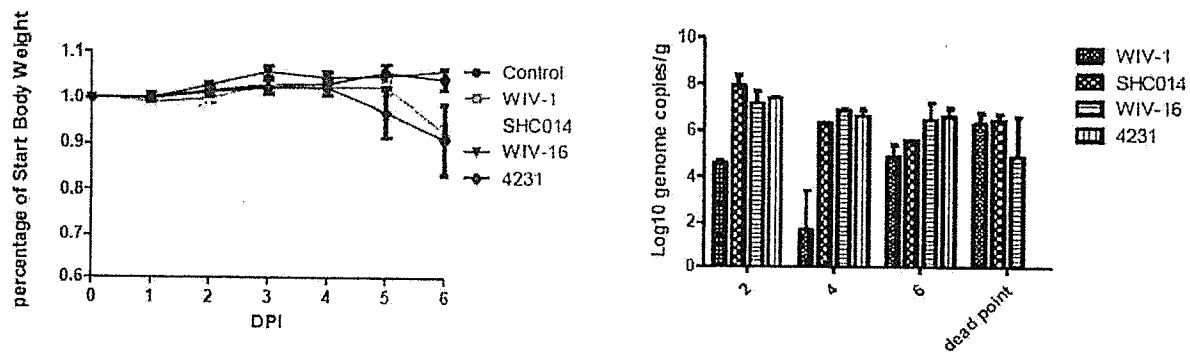


Figure 35. *In vivo* infection of SARSr-CoVs in hACE2-expressing mice. (a, left) Body weight change after infection; (b, right) Viral load in lung tissues

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.

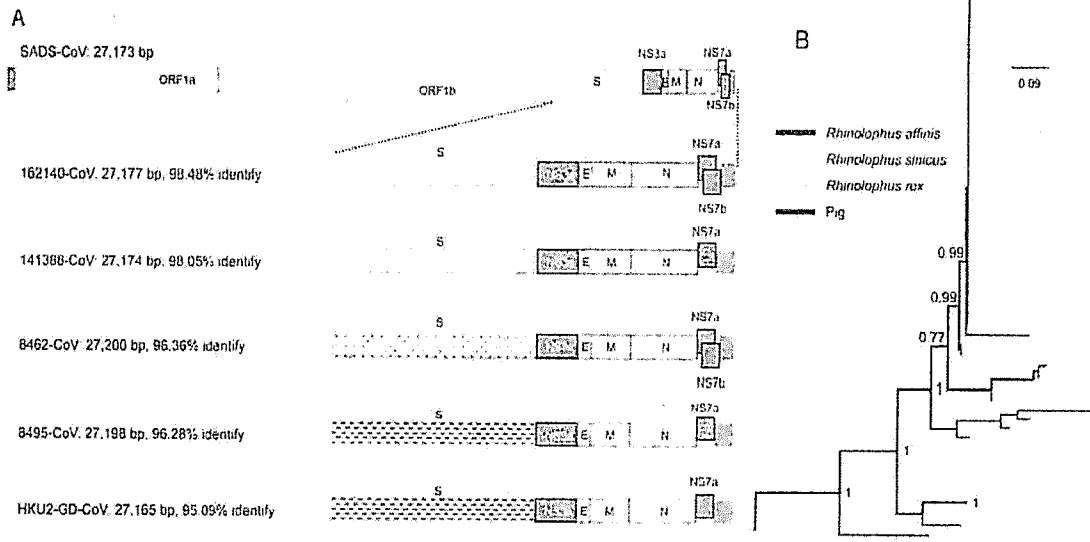


Figure 36. Genome organization and comparison (a) and Phylogenetic analysis of S1 protein (b) of SADS-CoV and bat SADSr-CoVs

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.

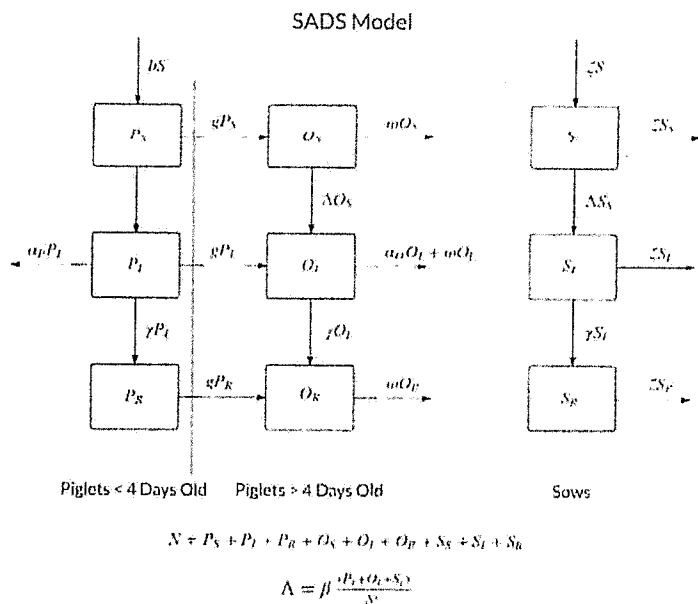


Figure 37: Schematic of intra-farm transmission mode.

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.

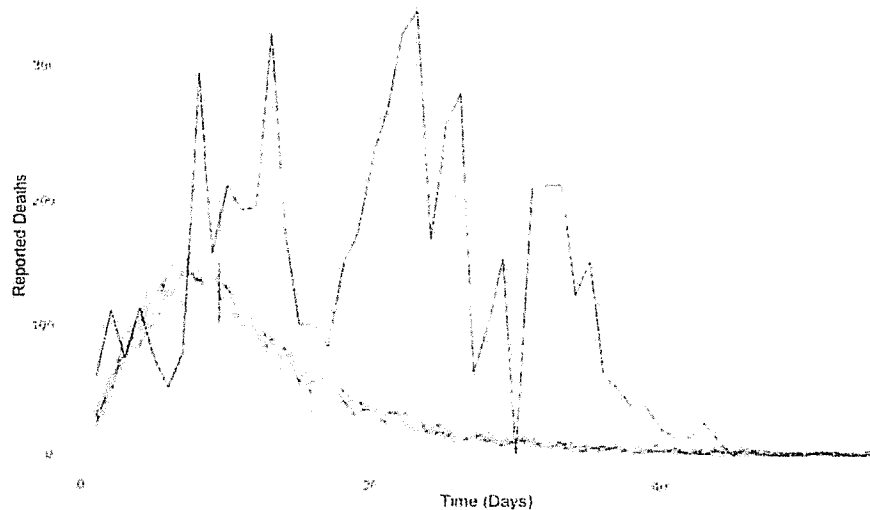


Figure 38: Best-fit simulations (red) from an equal-mixing transmission model and actual reported death time series (black) on a SADS-infected farm.

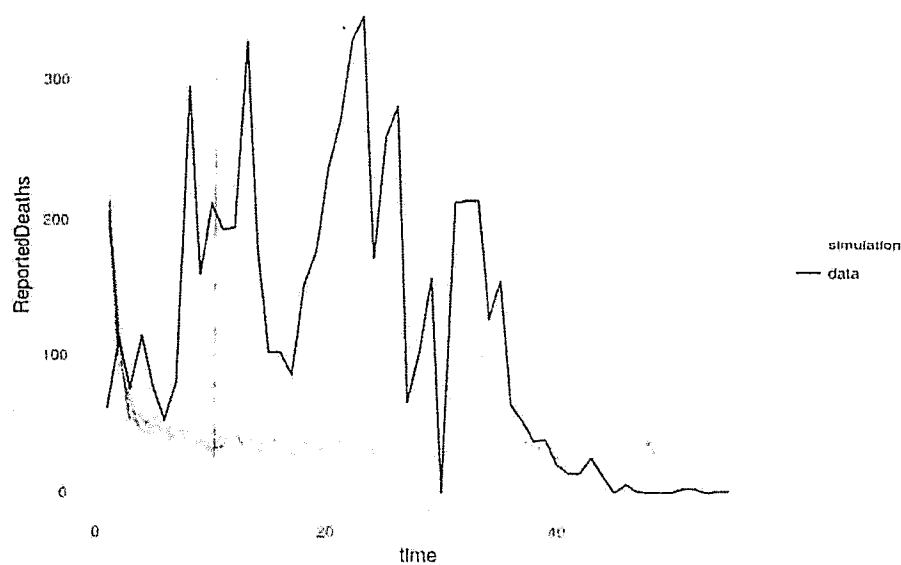


Figure 39: Best-fit simulations (grey) from an metapopulation transmission model and actual reported death time series (black) on a SADS-infected farm.

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.

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.

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	Olival KJ, Hosseini PR, Zambrana-Torrel C, Ross N, Bogich TL, Daszak P. Host and viral traits predict zoonotic spillover from mammals. <i>Nature</i> . 2017 June 29;546(7660):646-650. PubMed PMID: 28636590; PubMed Central PMCID: PMC5570460.
Complete	Hu B, Zeng LP, Yang XL, Ge XY, Zhang W, Li B, Xie JZ, Shen XR, Zhang YZ, Wang N, Luo DS, Zheng XS, Wang MN, Daszak P, Wang LF, Cui J, Shi ZL. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. <i>PLoS pathogens</i> . 2017 November;13(11):e1006698. PubMed PMID: 29190287; PubMed Central PMCID: PMC5708621.
In Process at NIHMS	Luo Y, Li B, Jiang RD, Hu BJ, Luo DS, Zhu GJ, Hu B, Liu HZ, Zhang YZ, Yang XL, Shi ZL. Longitudinal Surveillance of Betacoronaviruses in Fruit Bats in Yunnan Province, China During 2009-2016. <i>Virologica Sinica</i> . 2018 February;33(1):87-95. PubMed PMID: 29500692.
In Process at NIHMS	Wang N, Li SY, Yang XL, Huang HM, Zhang YJ, Guo H, Luo CM, Miller M, Zhu G, Chmura AA, Hagan E, Zhou JH, Zhang YZ, Wang LF, Daszak P, Shi ZL. Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China. <i>Virologica Sinica</i> . 2018 February;33(1):104-107. PubMed PMID: 29500691.
In Process at NIHMS	Zhou P, Fan H, Lan T, Yang XL, Shi WF, Zhang W, Zhu Y, Zhang YW, Xie QM, Mani S, Zheng XS, Li B, Li JM, Guo H, Pei GQ, An XP, Chen JW, Zhou L, Mai KJ, Wu ZX, Li D, Anderson DE, Zhang LB, Li SY, Mi ZQ, He TT, Cong F, Guo PJ, Huang R, Luo Y, Liu XL, Chen J, Huang Y, Sun Q, Zhang XL, Wang YY, Xing SZ, Chen YS, Sun Y, Li J, Daszak P, Wang LF, Shi ZL, Tong YG, Ma JY. Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. <i>Nature</i> . 2018 April;556(7700):255-258. PubMed PMID: 29618817.

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

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

Yes

Is the research exempt from Federal regulations?

No

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Report Attached: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

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

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

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

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional	Address
--------------------	------	---------------	---------

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

Organization Name: Wuhan School of Public Health

Country: CHINA

Description of Foreign Component:

Principal Coordinating Team for all project field work as per section G8 (above) and detailed in our Specific Aims

G.10 ESTIMATED UNOBLIGATED BALANCE

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

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

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

No

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 166195

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Foreign

NIH Defined Phase III Clinical Trial: No

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

Planned Enrollment

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
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
Unknown or Not Reported										
Total	1230	1230		0	0					2460

Cumulative Enrollment

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



APPROXIMATE WAIT TIME < 1 MINUTE

Project Information ?

2R01AI110964-06

- Description
- Details
- **RESULTS**
- History
- Subprojects

Project Number: 2R01AI110964-06

Contact PI / Project Leader: [DASZAK, PETER](#)

Title: UNDERSTANDING THE RISK OF BAT
CORONAVIRUS EMERGENCE

Awardee Organization: ECOHEALTH
ALLIANCE, INC.

Abstract Text:

Project Summary: Understanding the Risk of Bat Coronavirus Emergence Novel zoonotic, bat-origin CoVs are a significant threat to global health and food security, as the cause of SARS in China in 2002, the ongoing outbreak of MERS, and of a newly emerged Swine Acute Diarrhea Syndrome in China. In a previous R01 we found that bats in southern China harbor an extraordinary diversity of SARSr-CoVs, some of which can use human ACE2 to enter cells, infect humanized mouse models causing SARS-like illness, and evade available therapies or vaccines. We found that people living close to bat habitats are the primary risk groups for spillover, that at one site diverse SARSr-CoVs exist that contain every genetic element of the SARS-CoV genome, and identified serological evidence of human exposure among people living nearby. These findings have led to 18 published peer-reviewed papers, including two papers in Nature, and a review in Cell. Yet salient questions remain on the origin, diversity, capacity to cause illness, and risk of spillover of these viruses. In this R01 renewal we will address these issues through 3 specific aims: Aim 1. Characterize the diversity and distribution of high spillover-risk SARSr-CoVs in bats in southern China. We will use phylogeographic and viral discovery curve analyses to target additional bat sample collection and molecular CoV screening to fill in gaps in our previous sampling and fully characterize natural SARSr-CoV diversity in southern China. We will sequence receptor binding domains (spike proteins) to identify viruses with the highest potential for spillover which we will include in our experimental investigations (Aim 3). Aim 2. Community, and clinic-based syndromic,

surveillance to capture SARSr-CoV spillover, routes of exposure and potential public health consequences. We will conduct biological-behavioral surveillance in high-risk populations, with known bat contact, in community and clinical settings to 1) identify risk factors for serological and PCR evidence of bat SARSr-CoVs; & 2) assess possible health effects of SARSr-CoVs infection in people. We will analyze bat-CoV serology against human-wildlife contact and exposure data to quantify risk factors and health impacts of SARSr-CoV spillover. Aim 3. In vitro and in vivo characterization of SARSr-CoV spillover risk, coupled with spatial and phylogenetic analyses to identify the regions and viruses of public health concern. We will use S protein sequence data, infectious clone technology, in vitro and in vivo infection experiments and analysis of receptor binding to test the hypothesis that % divergence thresholds in S protein sequences predict spillover potential. We will combine these data with bat host distribution, viral diversity and phylogeny, human survey of risk behaviors and illness, and serology to identify SARSr-CoV spillover risk hotspots across southern China. Together these data and analyses will be critical for the future development of public health interventions and enhanced surveillance to prevent the re-emergence of SARS or the emergence of a novel SARSr-CoV.

Public Health Relevance Statement:

Program Director/Principal Investigator: Daszak, Peter
 Renewal: Understanding the Risk of Bat Coronavirus Emergence
 Project Narrative Most emerging human viruses come from wildlife, and these represent a significant threat to public health and biosecurity in the US and globally, as was demonstrated by the SARS coronavirus pandemic of 2002-03. This project seeks to understand what factors allow coronaviruses, including close relatives to SARS, to evolve and jump into the human population by studying viral diversity in their animal reservoirs (bats), surveying people that live in high-risk communities in China for evidence of bat-coronavirus infection, and conducting laboratory experiments to analyze and predict which newly-discovered viruses pose the greatest threat to human health.

NIH Spending Category:

Biodefense; Biotechnology; Clinical Research; Emerging Infectious Diseases; Infectious Diseases; Lung; Pneumonia; Pneumonia & Influenza; Prevention; Rare Diseases

Project Terms:

Acute; Acute Diarrhea; Address; Amino Acid Sequence; Animals; base; Behavior; Behavioral; Biological; biosecurity; Cells; China; Chiroptera; Clinic; Clinic Visits; Clinical; Communities; community clinic; Coronavirus; Coronavirus Infections; Coupled; Data; Data Analyses; Development; Disease Outbreaks; epidemiologic data; Epithelial Cells; experimental study; exposed human population; exposure route; Exposure to; Family suidae; follow-up; food security; Future; genetic element; Genome; Geographic Distribution; Geography; global health; Habitats; Health; high risk; high risk population; Human; human population study; humanized mouse; In Vitro; in vivo; Individual; Infection; Influenza; Investigation; laboratory experiment; Lead; Maps; Middle East Respiratory Syndrome Coronavirus; Modeling; Molecular; Monoclonal Antibodies; mouse model; Nature; novel; pandemic disease; Paper; Patients; Phylogenetic Analysis; Phylogeny; Prevalence; prevent; Principal Investigator; programs; Proteins; Public Health; public health intervention; Publishing Peer Reviews; Questionnaires; Readiness; Reagent; receptor binding; recombinant virus; respiratory; Risk; Risk Behaviors; Risk Factors; sample collection; Sampling; SARS coronavirus; screening; Serologic tests; Serological; seropositive; Severe Acute Respiratory Syndrome; Site; Surveys; Syndrome; syndromic surveillance; Technology; Testing; Therapeutic Intervention; Therapeutic Monoclonal Antibodies; therapeutic vaccine; Time; trait; Transgenic Organisms; Vaccines; Viral; virology; Virus; Work; Zoonoses

Contact PI Information:	Program Official Information:	Other PI Information:
Name: DASZAK, PETER Email: Click to view contact PI email address Title: PRESIDENT	Name: STEMMY, ERIK J Email: Click to view PO email address	Not Applicable

Organization:	Department / Educational Institution Type:	Congressional District:
Name: ECOHEALTH ALLIANCE, INC.	Unavailable	State Code: NY
City: NEW YORK Country: UNITED STATES (US)	Unavailable	District: 10

Other Information:		
FOA: PA-18-484	DUNS Number: 077090066	CFDA Code: 855
Study Section: Clinical Research and Field Studies of Infectious Diseases Study Section (CRFS)	Project Start Date: 1-JUN-2014	Project End Date: 30-JUN-2025
Fiscal Year: 2019 Award Notice Date: 24-JUL-2019	Budget Start Date: 24-JUL-2019	Budget End Date: 30-JUN-2021

Administering Institutes or Centers:
 NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Project Funding Information for 2019:		
Total Funding: \$661,980	Direct Costs: \$538,926	Indirect Costs: \$123,054

Year	Funding IC	FY Total Cost by IC
2019	NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES	\$661,980

Categorical Spending by IC: [Click here for more information on NIH Categorical Spending](#)
 Biodefense; Biotechnology; Clinical Research; Emerging Infectious Diseases; Infectious Diseases; Lung; Pneumonia; Pneumonia & Influenza; Prevention; Rare Diseases;

NIH Categorical Spending:		
Funding IC	FY Total Cost by IC	NIH Spending Category
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES	\$661,980	Pneumonia; Lung; Rare Diseases; Biotechnology; Infectious Diseases; Biodefense; Prevention; Clinical Research; Emerging Infectious Diseases; Pneumonia & Influenza;

History:
 Total project funding amount for 6 projects is \$3,748,715*
 * Only NIH, CDC, and FDA funding data.

Project Number	Sub #	Project Title	Contact Principal Investigator	Organization	FY	Admin IC	Funding IC	FY Total Cost by IC
2R01AI110964-06		UNDERSTANDING THE RISK OF BAT CORONAVIRUS EMERGENCE	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.	2019	NIAID	NIAID	\$661,980
5R01AI110964-05		UNDERSTANDING THE RISK OF BAT	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.	2018	NIAID	NIAID	\$581,646

CORONAVIRUS
EMERGENCE

5R01AI110964-04	UNDERSTANDING THE RISK OF BAT CORONAVIRUS EMERGENCE	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.	2017 NIAID	NIAID	\$597,112
5R01AI110964-03	UNDERSTANDING THE RISK OF BAT CORONAVIRUS EMERGENCE	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.	2016 NIAID	NIAID	\$611,090
5R01AI110964-02	UNDERSTANDING THE RISK OF BAT CORONAVIRUS EMERGENCE	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.	2015 NIAID	NIAID	\$630,445
1R01AI110964-01	UNDERSTANDING THE RISK OF BAT CORONAVIRUS EMERGENCE	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.	2014 NIAID	NIAID	\$666,442

Subprojects:

Project Number	Sub #	Project Title	Contact Principal Investigator	Organization	FY	Admin IC	FY Total Cost by IC
----------------	-------	---------------	--------------------------------------	--------------	----	-------------	------------------------------

No Subprojects information available for 2R01AI110964-06