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: ofxpo ange and in bate (s and ot)-1 (h)1 (e)-1 (r)-1 (m)1 (ammal)1 (s)-el1 (xam)-1 (ted by and ot)-1 (h)
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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 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, Puerta Vallerta, 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 m( )-1 (m1 (,)1 (s)-1 (o)1 (f)-1 (Memu1. Pub,)1 (hu1. Pub,)1 (hu1

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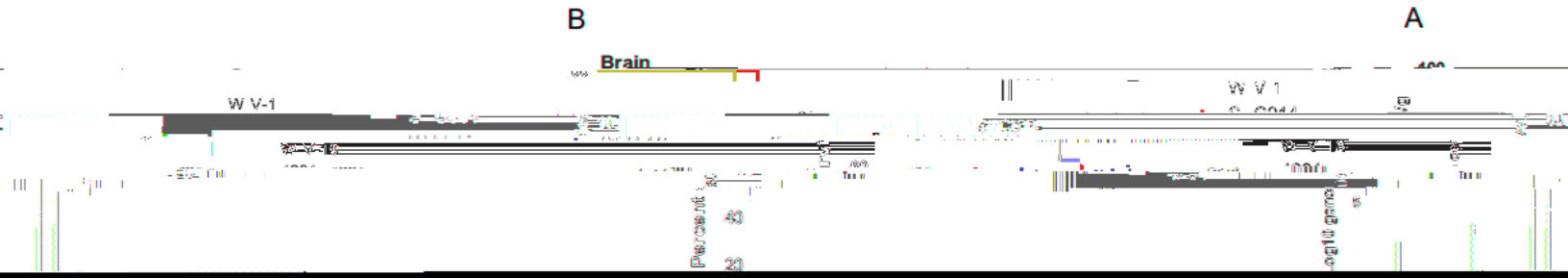
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C. PRODUCTS

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

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
	Y	DASZAK, PETER	BS,PHD	PD/PI						NA
	N	KE, CHANGWEN	PHD	Co-Investigator				Center for Disease Control and Prevention of Guangdon g 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
	N	Chmura, Aleksei								

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 INFRASTRUCT

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
$66,500	CHINA

Description of Foreign Component:

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 * m Yes l No

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

1.4. Clinical Trial Questionnaire *

1.4.a. Does the study involve human participants?	l Yes	m No
1.4.b. Are the participants prospectively assigned to an intervention?	m Yes	l No
1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?	m Yes	l No
1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?	m Yes	l No

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

Section 2 - Study Population Characteristics (Study 58010)

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Section 3 - Protection and Monitoring Plans (Study 58010)

m Yes m No m N/A

If yes, describe the single IRB plan

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Section 4 - Protocol Synopsis (Study 58010)

Type	Name	Description

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

4.1.f. Masking m Yes m No

o Participant o Care Provider o Investigator o Outcomes Assessor

4.1.g. Allocation

Type	Name	Time Frame	Brief Description

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

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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 dentified 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

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Year 4 Report: Understanding the Risk of Bat Coronavirus Emergence

Award Number : R01AI110964-03

Reporting Period : 06/01/2017 ±05/31/2018

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B.2 What was accomplished under these goals?

Summary

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).

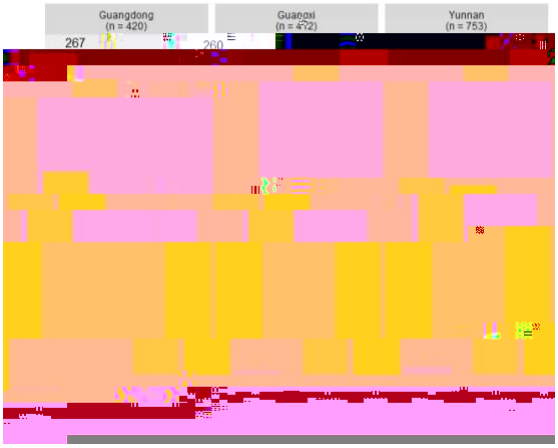


Figure 1: *Gender of respondents*

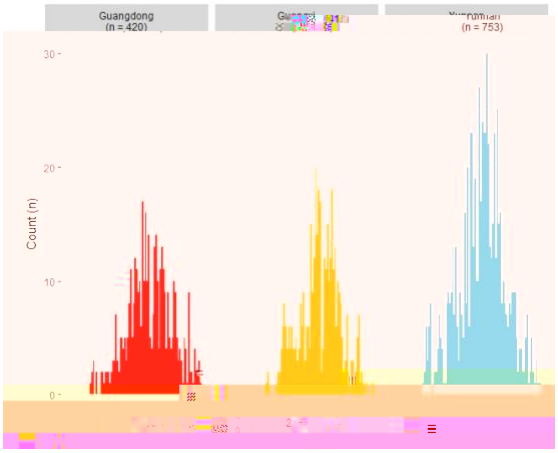


Figure 2: *Age distribution of respondents.*

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%).

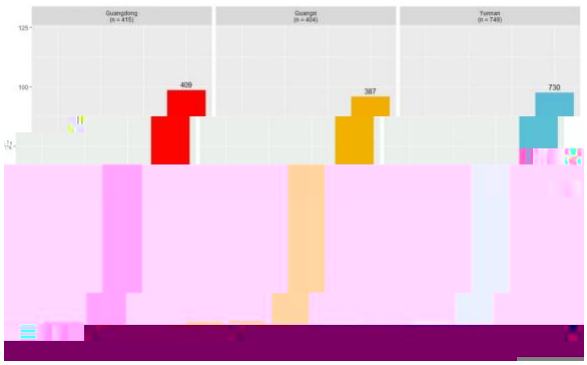


Figure 3 : *Duration of residency.*

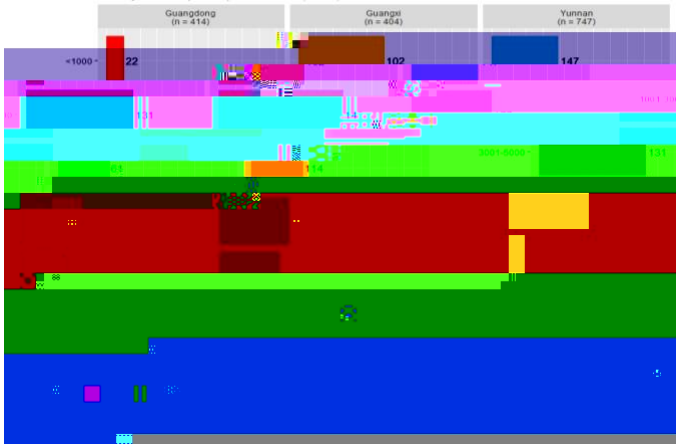
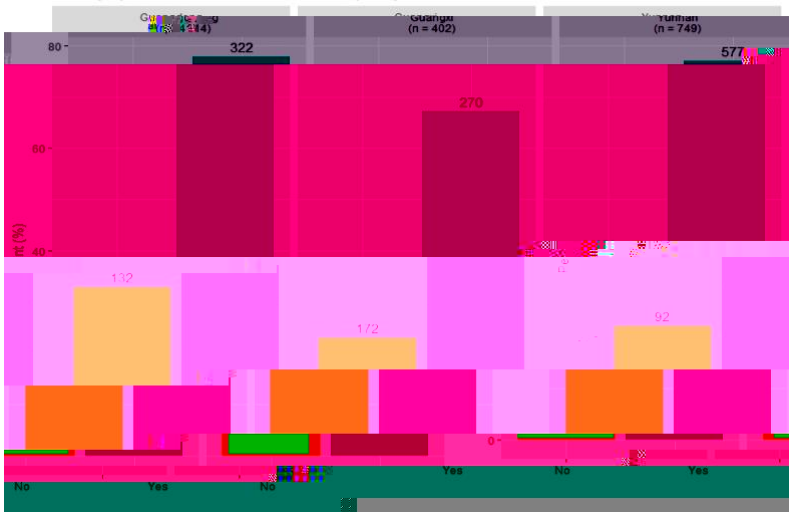
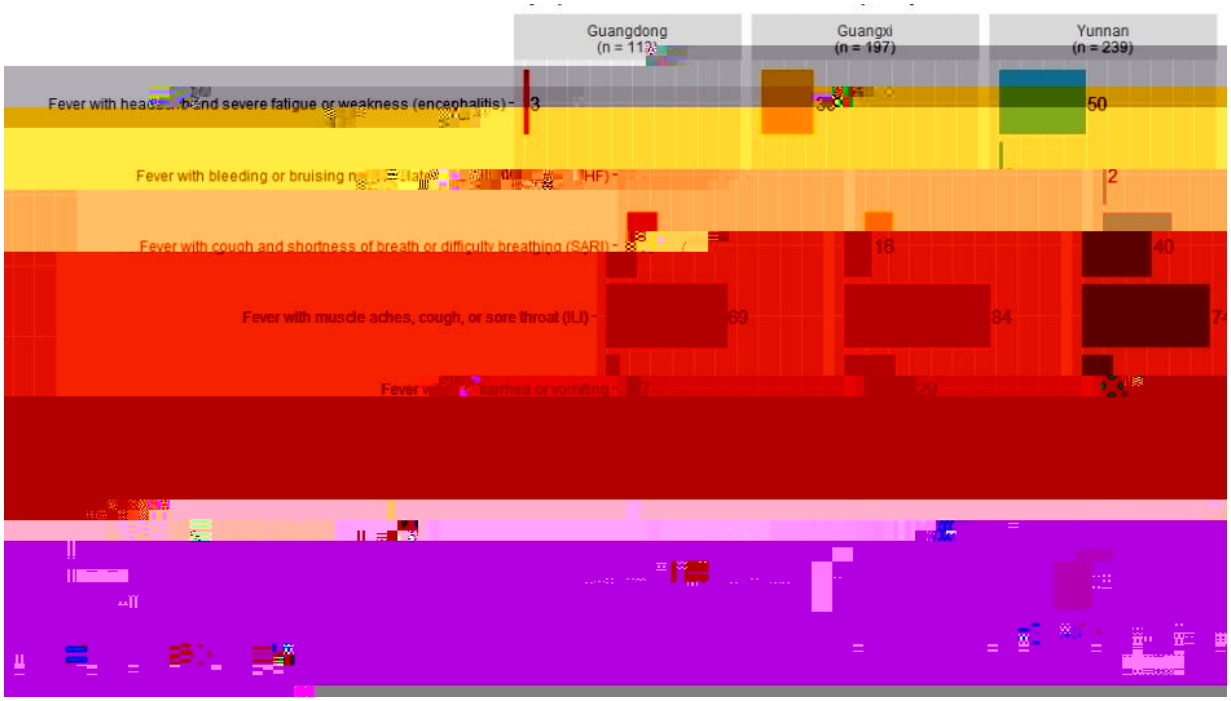


Figure 4: *Family annual per capita income (RMB).*

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

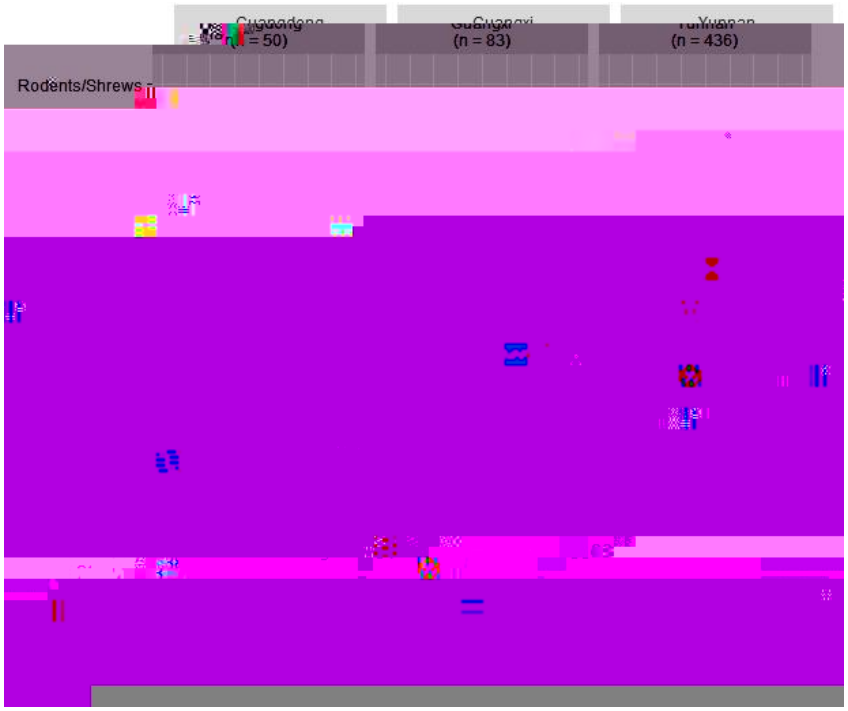


Of the respondents who reported having symptoms of unusual illness in the past year, across all three provinces, symptoms associated ILI were the most commonly reported. In Guangdong province, this was followed by symptoms associated with SARI then by other symptoms not provided in the survey. In Guangxi,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



Contact with Animals

All respondents were asked about various types of animal co



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).

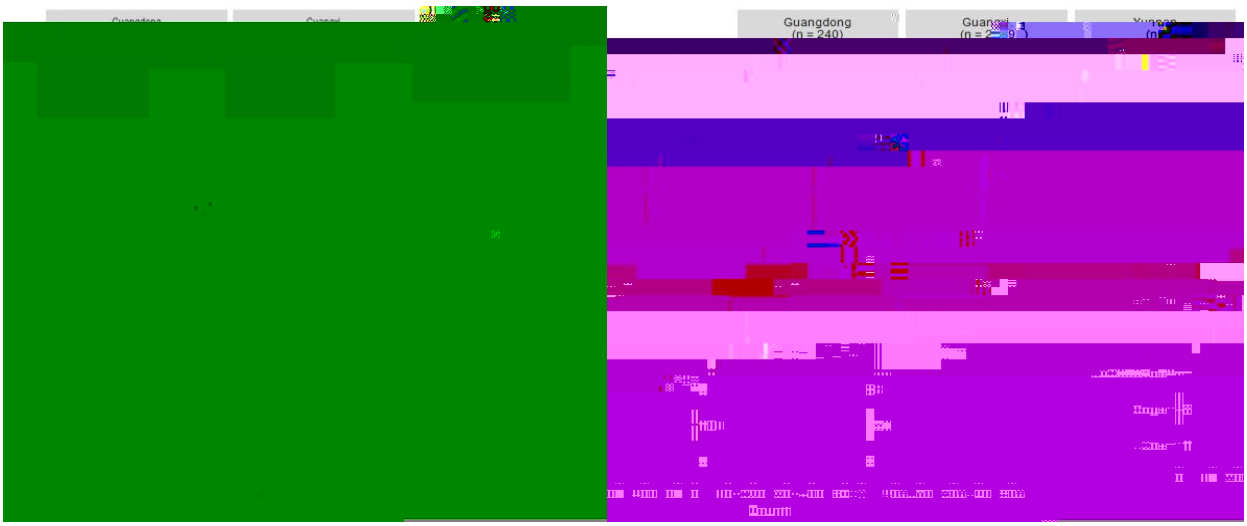
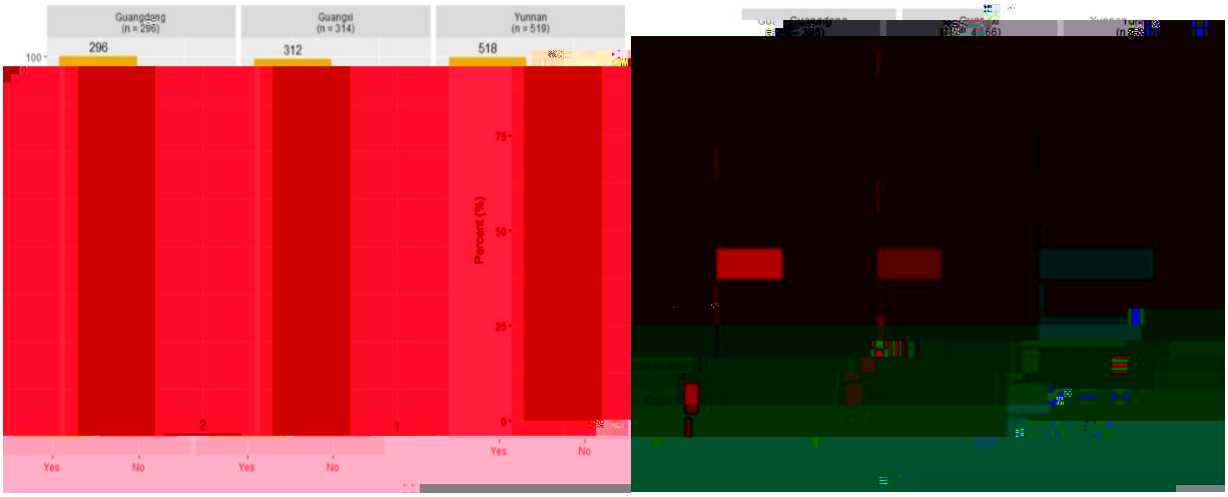


Figure 17 a & b: *(a) Whether respondents had handled live animals, in the past year, and (b) among those who had, types of live animals handled.*

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 18 a & 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).

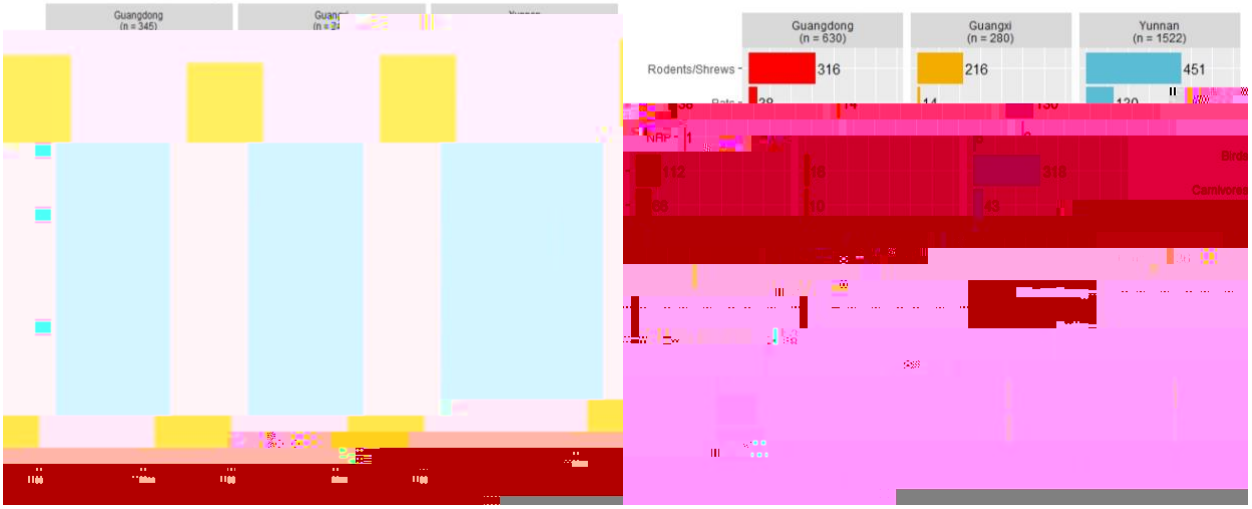
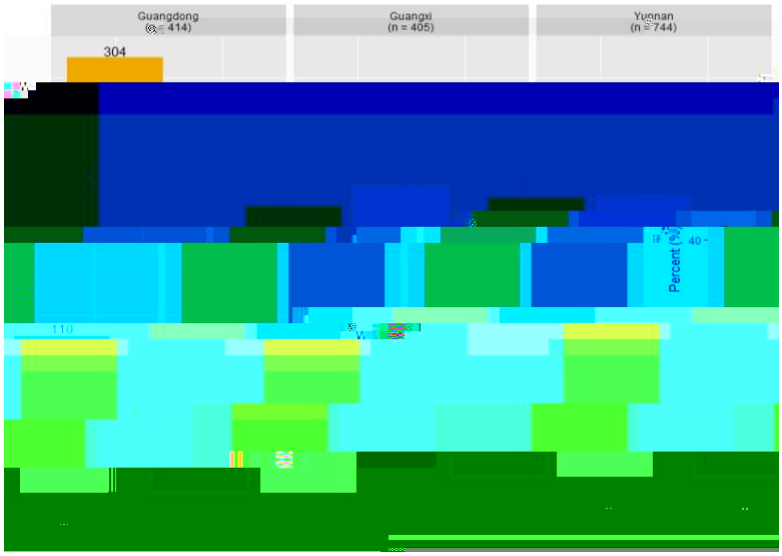


Figure 19 a & b: *(a) Whether respondents had animals come inside dwelling, in the past year, and (b) among those who had, types of animals in dwelling.*


Guangdong
(n = 414)
Guangxi
(n = 405)
Yunnan
(n = 744)
304
Percent (%)
40
110

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)

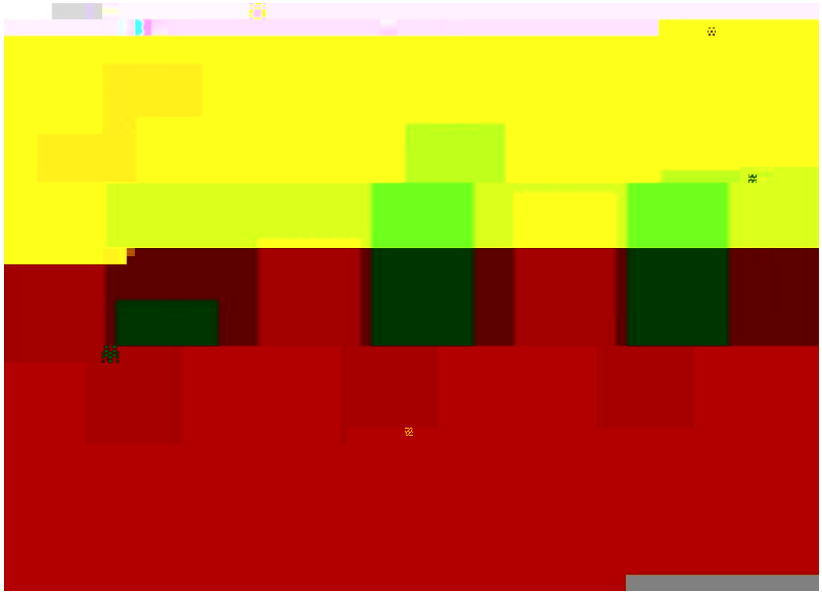


Figure 28 : *Whether respondents were worried about diseases or disease outbreaks in animals at wet markets.*

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).

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

Figure 29 :

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

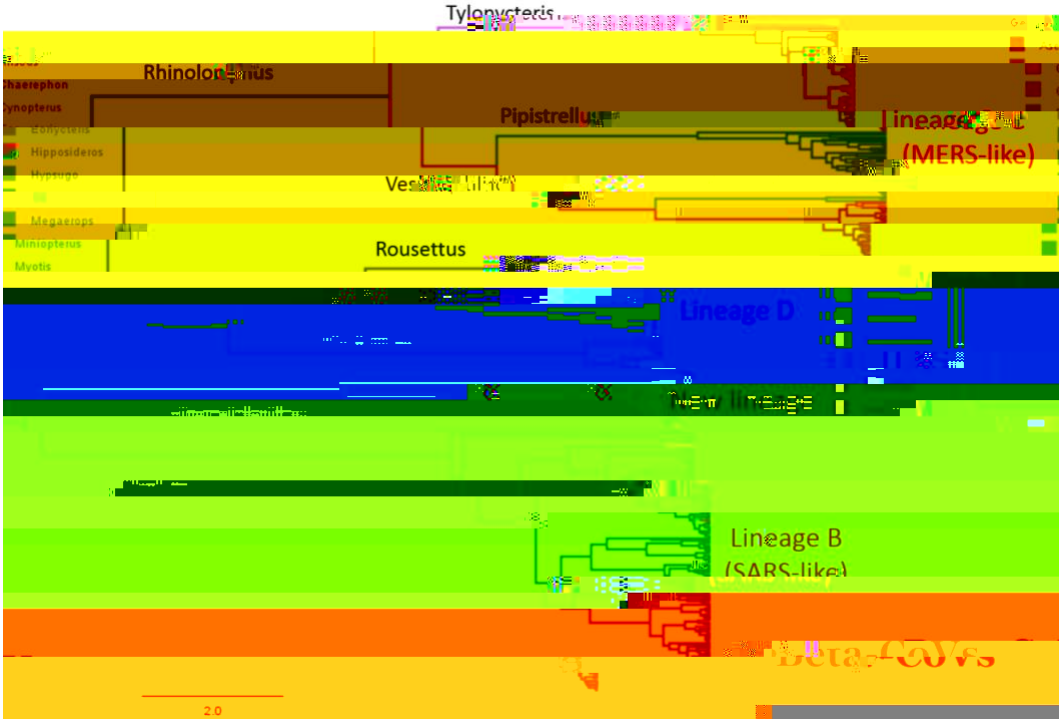


Figure 31 . *Ancestral host reconstruction for Beta-CoVs, at a host genus level.*

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

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

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

SHC014
Control

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

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

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* F &OLQLFDO7ULDOV JRY 'RHV WKLV SURMHFW LQFOXGH RQH RU PRUH DSSOLFDEOH FOLQLFDO WULDOV WKDW 1R
* +80$1 68%-(&76 ('8&$7,21 5(48,5(0(17 $UH WKHUH SHUVRQQHO RQ WKLV SURMHFW ZKR DUH QHZO\ LQYROYHG LQ WKH GHVL 1R
* +80$1 (0%5<21,& 67(0 &(//6 +(6&6 'RHV WKLV SURMHFW LQYROYH KXPDQ HPEU\RQLF VWHP FHOOV RQO\ K(6& OLQHV OL IXQGHG UHVHDUFK " 1R
* 9(57(%5$7($1,0$/6 'RHV WKLV SURMHFW LQYROYH YHUWHEUDWH DQLPDOV" <HV
* 352-(&7 3(5)250$1&(6,7(6

2UJDQL]DWLRQ 1DPH	'8166RQJUHVVLRQDO $GGUHVV

		'LVWULFW	
3ULPDU (FR+HDOWK $OOLDQFH ,QF		1<	:HVW WK 6WUHHW WK)ORRU 1HZ <RUN 1<
:XKDQ ,QVWLWXWH RI 9LURORJ\			;LDR +RQJ 6KDQ 1R :XFKDQJ 'LVWULFW :XKDQ
(DVW &KLQD 1RUPDO 8QLYHUVLW\			=KRQJVKDQ %HLOX 6KDQJKDL

***)25(,*1 &20321(17**

2UJDQL]DWLRQ 1DPH :XKDQ ,QVWLWXWH RI 9LURORJ\
&RXQWU &+,1$
'HVFULSWLRQ RI)RUHLJQ &RPSRQHQW
3ULQFLSDO /DERUDWRU\ IRU DOO 5HVHDUFK LQ &KLQD DV SHU VHFWLRQ * DERYH D

2UJDQL]DWLRQ 1DPH :XKDQ 6FKRRO RI 3XEOLF +HDOWK
&RXQWU &+,1$
'HVFULSWLRQ RI)RUHLJQ &RPSRQHQW
3ULQFLSDO &RRUGLQDWLQJ 7HDP IRU DOO SURMHFW ILHOG ZRUN DV SHU VHFWLRQ *

*** (67,0$7(' 812%/,*$7(' %$/$1&(**

*** D ,V LW DQWLFLSDWHG WKDW DQ HVWLPDWHG XQREOLJDWHG EDODQFH LQFOXGL \HU V WRWDO DSSURYHG EXGJHW"**

1R

*** 352*5$0 ,1&20(**

,V SURJUDP LQFRPH DQWLFLSDWHG GXULQJ WKH QH[W EXGJHW SHULRG"

1R

***) $ &2676**

,V WKHUH D FKDQJH LQ SHUIRUPDQFH VLWHV WKDW ZLOO DIIHFW) $ FRVWV"

1R

,QFOXVLRQ (QUROOPHQW 5HSRUW

,QFOXVLRQ 'DWD 5HFRUG ,'5 8VLQJ DQ ([LVWLQJ 'DWDVHW RU 5HVRXUFH

'HOHWHG 2QVHW 6WXG\ " 1R &OLQLFDO 7ULDO 1R

(QUROOPHQW /RFDWLRQ)RUHLJQ 1,+ 'HILQHG 3KDVH ,,, &OLQLFDO 7ULDO 1R

6WXG\ 7LWOH 8QGHUVWDQGLQJ WKH 5LVN RI %DW &RURQDYLUXV (PHUJHQFH 35272&2

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