Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 06/18/2018

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI110964-05
FAIN: R01AI110964

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Understanding the Risk of Bat Coronavirus Emergence

Aleksei Chmura
President
460 West 34th Street
17th Floor
New York, NY 100012317

Award e-mailed to: (b) (6)

Period Of Performance:
Budget Period: 06/01/2018 – 05/31/2019
Project Period: 06/01/2014 – 05/31/2019

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $581,646 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to ECOHEALTH ALLIANCE, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI110964. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Tseday G Girma
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI110964-05

Award Calculation (U.S. Dollars)

Salaries and Wages	$167,708
Fringe Benefits	$54,168
Personnel Costs (Subtotal)	$221,876
Materials & Supplies	$3,500
Travel	$35,918
Other	$9,400
Subawards/Consortium/Contractual Costs	$191,576
Federal Direct Costs	$462,270
Federal F&A Costs	$119,376
Approved Budget	$581,646
Total Amount of Federal Funds Obligated (Federal Share)	$581,646
TOTAL FEDERAL AWARD AMOUNT	$581,646
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$581,646

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
5	$581,646	$581,646

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1311726494A1
Document Number:	RAI110964A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2018

IC	CAN	2018
AI	8472350	$581,646

NIH Administrative Data:
PCC: M51C / **OC**: 414E / **Released**: (b) (6) 06/15/2018
Award Processed: 06/18/2018 12:02:35 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI110964-05

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI110964-05

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI110964. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/policy.htm#gps.

A final expenditure Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 120 days of the period of performance end date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/policy.htm#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) quarterly cash transaction data. A final quarterly federal cash transaction report is not required for awards in PMS B subaccounts (i.e., awards to foreign entities and to Federal agencies). NIH will close the awards using the last recorded cash drawdown level in PMS for awards that do not require a final FFR on expenditures or quarterly federal cash transaction reporting. It is important to note that for financial closeout, if a grantee fails to submit a required final expenditure FFR, NIH will close the grant using the last recorded cash drawdown level. If the grantee submits a final expenditure FFR but does not reconcile any discrepancies between expenditures reported on the final expenditure FFR and the last cash report to PMS, NIH will close the award at the lower amount. This could be considered a debt or result in disallowed costs.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 120 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm. This paragraph does not apply to Training grants, Fellowships, and certain other programs—i.e., activity codes C06, D42, D43, D71, DP7, G07, G08, G11, K12, K16, K30, P09, P40, P41, P51, R13, R25, R28, R30, R90, RL5, RL9, S10, S14, S15, U13, U14, U41, U42, U45, UC6, UC7, UR2, X01, X02.

Unless an application for competitive renewal is submitted, a Final Research Performance Progress Report (Final RPPR) must also be submitted within 120 days of the period of performance end date. If a competitive renewal application is submitted prior to that date, then an Interim RPPR must be submitted by that date as well. Instructions for preparing an Interim or Final RPPR are at: https://grants.nih.gov/grants/rppr/rppr_instruction_guide.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the Interim or Final RPPR. *Note that data reported within Section I of the Interim and Final RPPR forms will be made public and should be written for a lay person audience.*

NIH strongly encourages electronic submission of the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final invention statement may be e-mailed as PDF attachments to: NIHCloseoutCenter@mail.nih.gov.

Hard copy: Paper submissions of the final invention statement may be faxed to the NIH Division of Central Grants Processing, Grants Closeout Center, at 301-480-2304, or mailed to:

National Institutes of Health
Office of Extramural Research
Division of Central Grants Processing
Grants Closeout Center
6705 Rockledge Drive
Suite 5016, MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express deliveries only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final RPPR is not required. However, a final expenditure FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI110964-05

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

If any experiments proposed in this award result in a virus with enhanced growth by more than 1 log compared to wild type strains, you must notify your NIAID Program Officer and Grants Management Specialist immediately. Further research involving the resulting virus(es) may require review by the Department of Health and Human Services in accordance with the Framework for Guiding Funding Decisions about Proposed Research Involving Enhanced Potential Pandemic Pathogens (https://www.phe.gov/s3/dualuse/Documents/P3CO.pdf).

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The Research Performance Progress Report (RPPR), Section G.9 (Foreign component), includes reporting requirements for all research performed outside of the United States. Research conducted at the following site(s) must be reported in your RPPR:

San Pya Clinic, BURMA
Institut Pasteur du Cambodge, CAMBODIA
Primate Research Center at Bogor Agricultural University, INDONESIA
Conservation Medicine, Ltd, MALAYSIA
King Chulalongkorn Memorial Hospital, THAILAND
Hanoi Agricultural University, VIETNAM
National Animal Health Laboratory, LAOS

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This Notice of Award (NoA) includes collaboration with **Wuhan University School of Public Health, CHINA.**

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This Notice of Award (NoA) includes funds for activity with **Wuhan Institute of Virology, CHINA.**

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This Notice of Award (NoA) includes funds for activity with **East China Normal University.**

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This award may include collaborations with and/or between foreign organizations. Please be advised that short term travel visa expenses are an allowable expense on this grant, if justified as critical and necessary for the conduct of the project.

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This award is subject to the Clinical Terms of Award included in Monitoring of Clinical Trials and Studies - NIAID (see NIH Guide for Grants and Contracts, July 8, 2002, NOT AI-02-032). These terms and conditions are hereby incorporated by reference, and can be accessed via the following World Wide Web address: https://www.niaid.nih.gov/grants-contracts/niaid-clinical-terms-award All submissions required by the NIAID Clinical Terms of Award must be forwarded electronically or by mail to the responsible NIAID Program Official identified on this Notice of Award.

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Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Adam Graham
Email: (b) (6) **Phone**: (b) (6) **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: (b) (6) **Phone**: (b) (6)

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI110964-05

INSTITUTION: ECOHEALTH ALLIANCE, INC.

Budget	Year 5
Salaries and Wages	$167,708
Fringe Benefits	$54,168
Personnel Costs (Subtotal)	$221,876
Materials & Supplies	$3,500
Travel	$35,918
Other	$9,400
Subawards/Consortium/Contractual Costs	$191,576
TOTAL FEDERAL DC	$462,270
TOTAL FEDERAL F&A	$119,376
TOTAL COST	$581,646

Facilities and Administrative Costs	Year 5
F&A Cost Rate 1	44.1%
F&A Cost Base 1	$270,694

F&A Costs 1	$119,376

A. COVER PAGE

Project Title: Understanding the Risk of Bat Coronavirus Emergence	
Grant Number: 5R01AI110964-05	**Project/Grant Period:** 06/01/2014 - 05/31/2019
Reporting Period: 06/01/2017 - 05/31/2018	**Requested Budget Period:** 06/01/2018 - 05/31/2019
Report Term Frequency: Annual	**Date Submitted:** 09/16/2020
Program Director/Principal Investigator Information: 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 hACE-expressing transgenic mice (survival rate, histopathological analysis, etc) to evaluate the risk of cross-species infection of different SARSr-CoVs to humans;
•Continue searching for the receptor of SARSr-CoVs with deletions in the homologous region of SARS-CoV RBD (i.e. Rp3, Rs672), and SARSr-CoVs that are unable to utilize bat ACE2 (e.g. Rs4231).
•Continue the phylogeographic study of bat-CoV with newly collected samples to better understand the geographic distribution and evolution of bat-CoV genetic diversity in south China and SE Asia.

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

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

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

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)**.

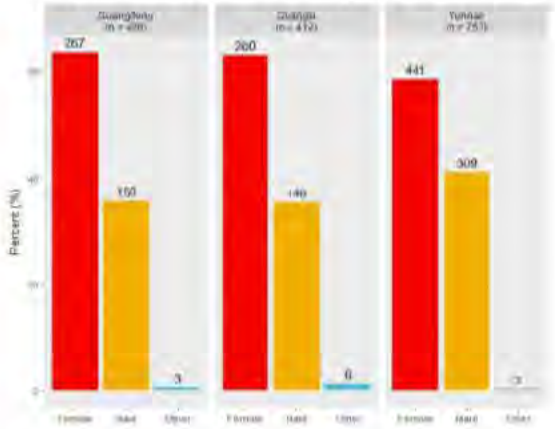


Figure 1: *Gender of respondents*

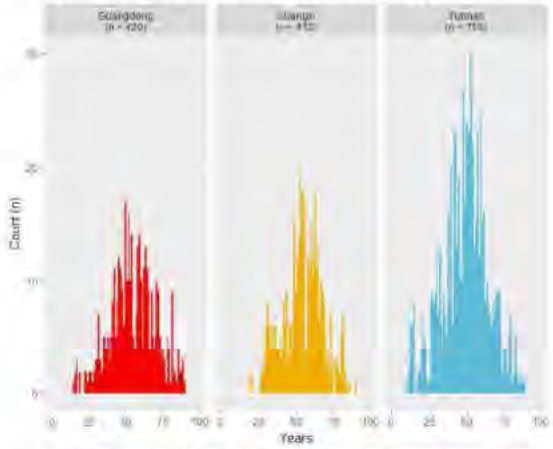


Figure 2: *Age distribution of respondents.*

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%).

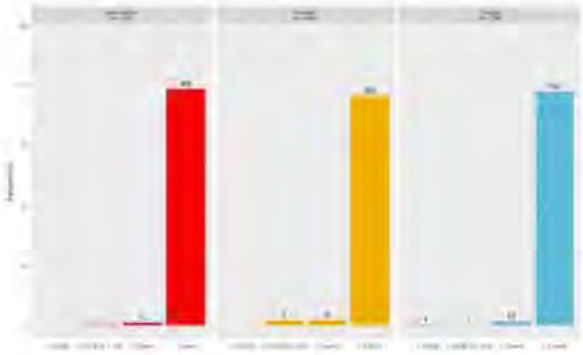


Figure 3: *Duration of residency.*

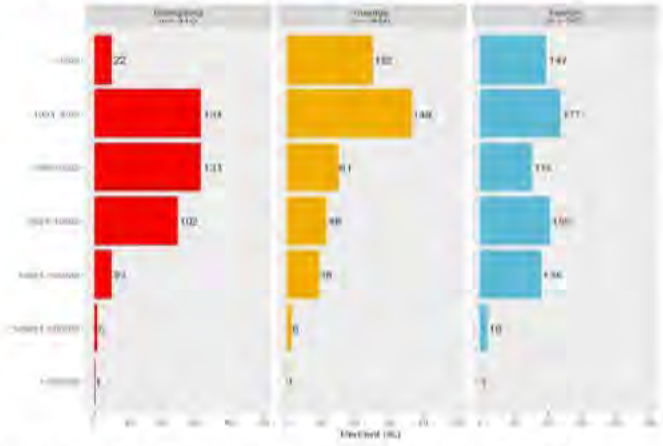


Figure 4: *Family annual per capita income (RMB).*

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

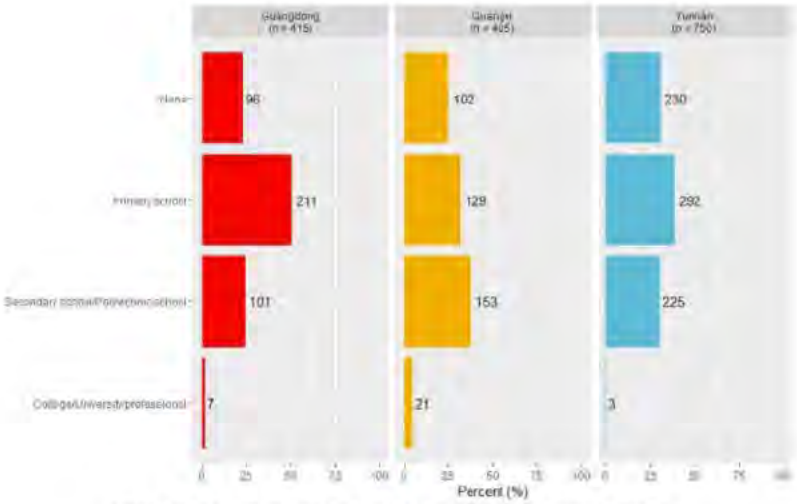


Figure 5: *Highest level of education completed*

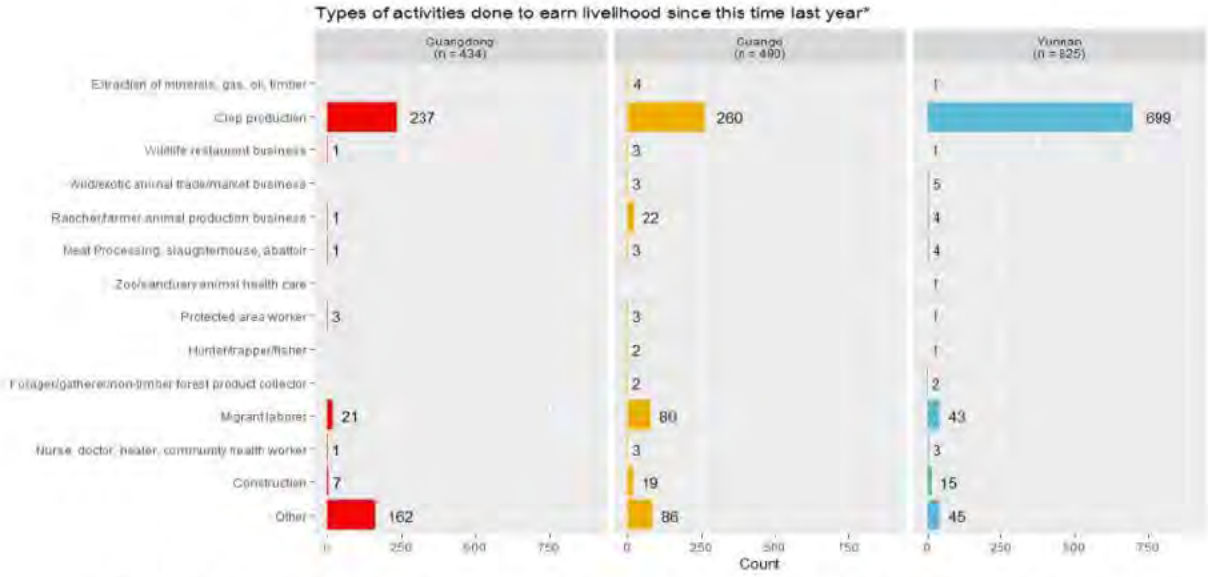


Figure 6: *Types of activities conducted to earn a livelihood since this time last year (above)*

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.

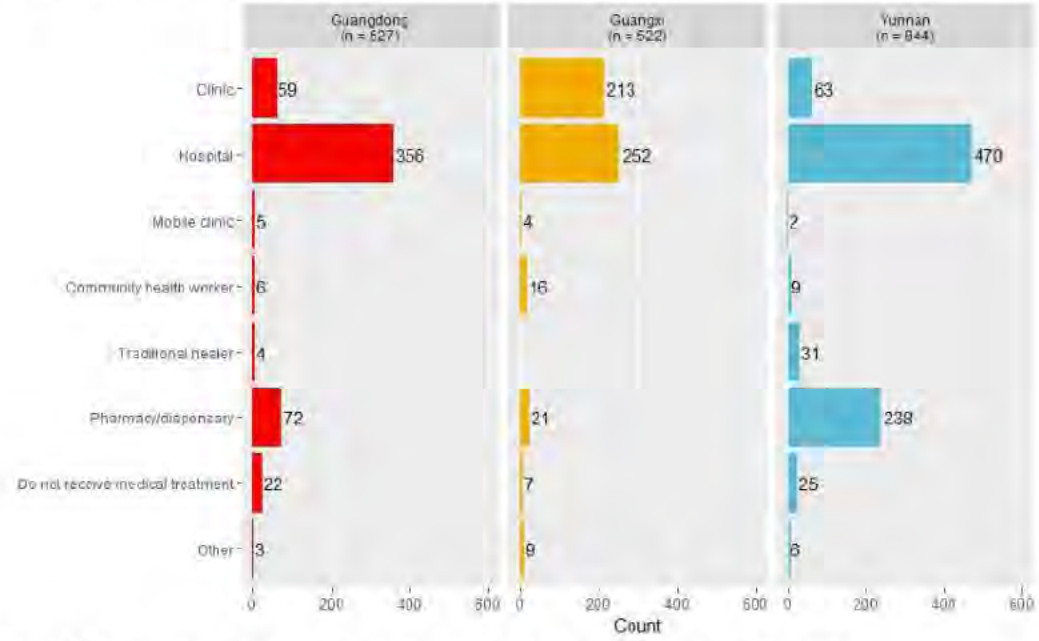


Figure 7: *Location where care was usually received for illness or infection.*

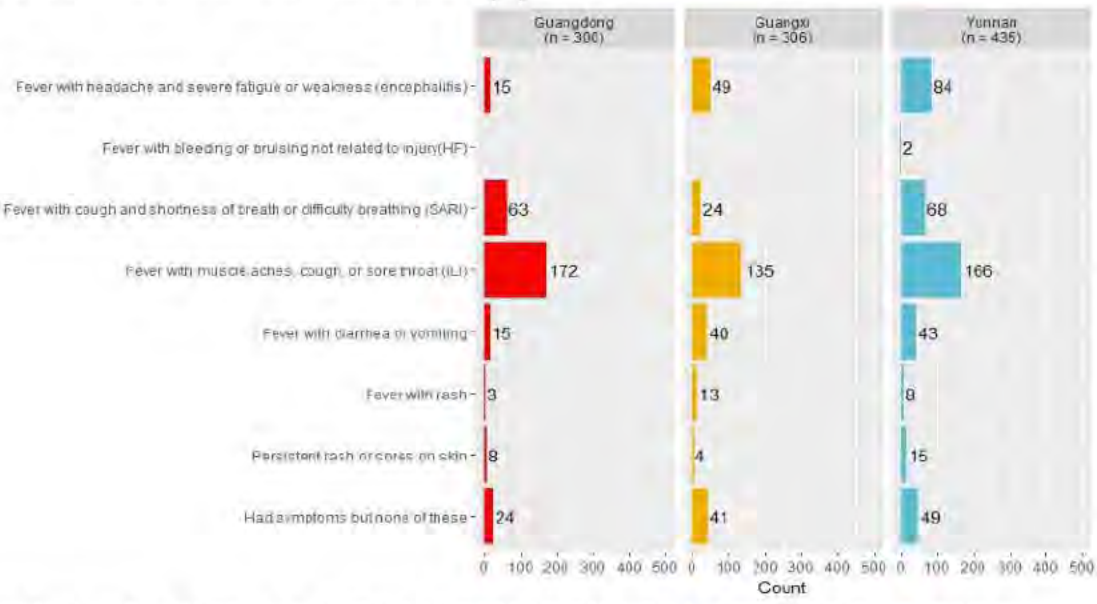
All survey respondents were asked whether they had experienced an unusual illness in their lifetime and in the past year, defined by a series of the most common symptoms associated with encephalitis, hemorrhagic fever (HF), severe acute respiratory infection (SARI), and influenza-like illness (ILI). 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.*

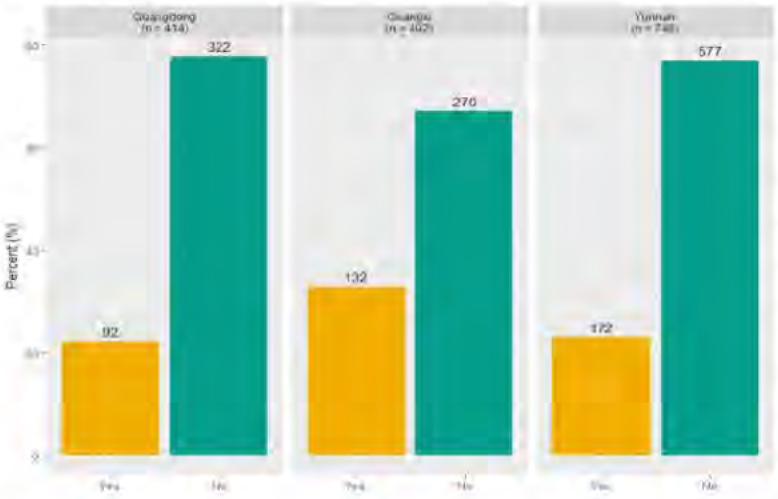
Across all three provinces, among those who had experienced any symptoms of unusual illness in their lifetimes, those associated with ILI were the most commonly reported. In Guangdong province, this was followed by symptoms associated with SARI, then by other symptoms not mentioned in the survey. In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



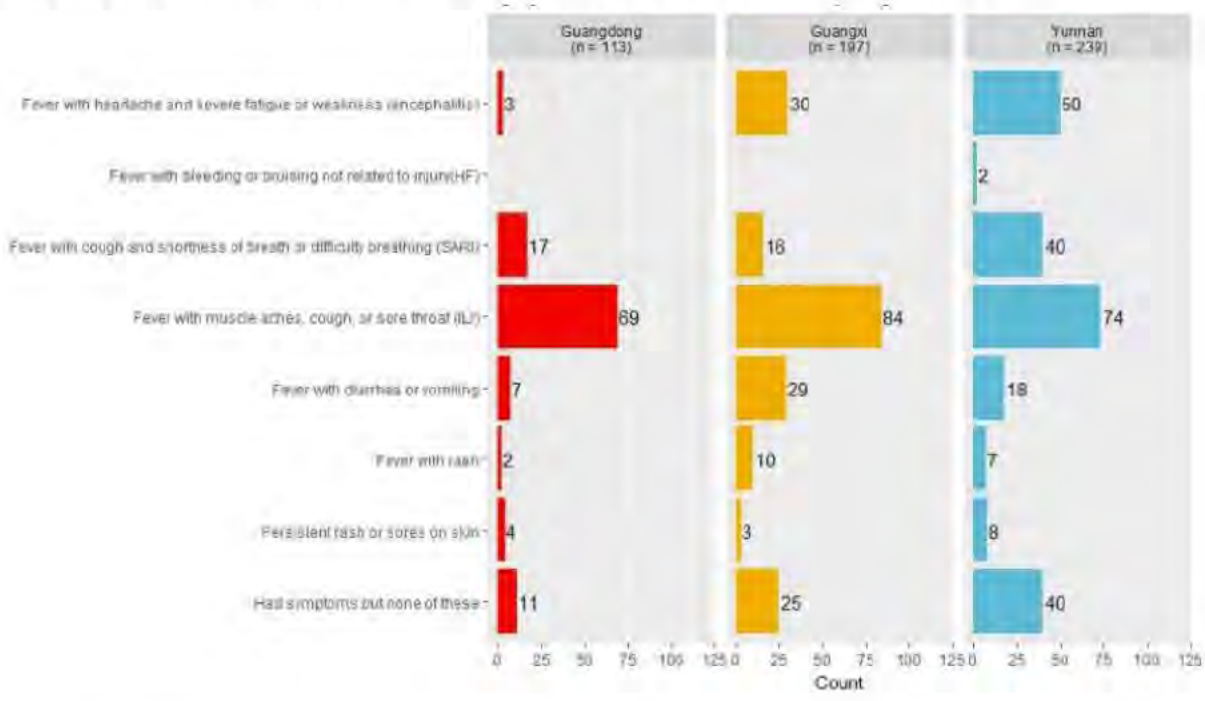
In each province, just under one-third of respondents who experienced the symptoms associated with an unusual illness in their lifetime indicated experiencing any of the symptoms in the past year – 22.2% in Guangdong, 32.8% in Guangxi and 23.0% in Yunnan **(Fig. 10)**.

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 ILI were the most commonly reported. In Guangdong province, this was followed by symptoms associated with SARI then by other symptoms not provided in the survey. In Guangxi, symptoms associated with ILI were followed by symptoms associated with encephalitis, then by fever with diarrhea or vomiting. In Yunnan, symptoms associated with ILI were followed by symptoms associated with encephalitis, then by both SARI and other symptoms not provided in survey **(Fig. 11)**.

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



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

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.

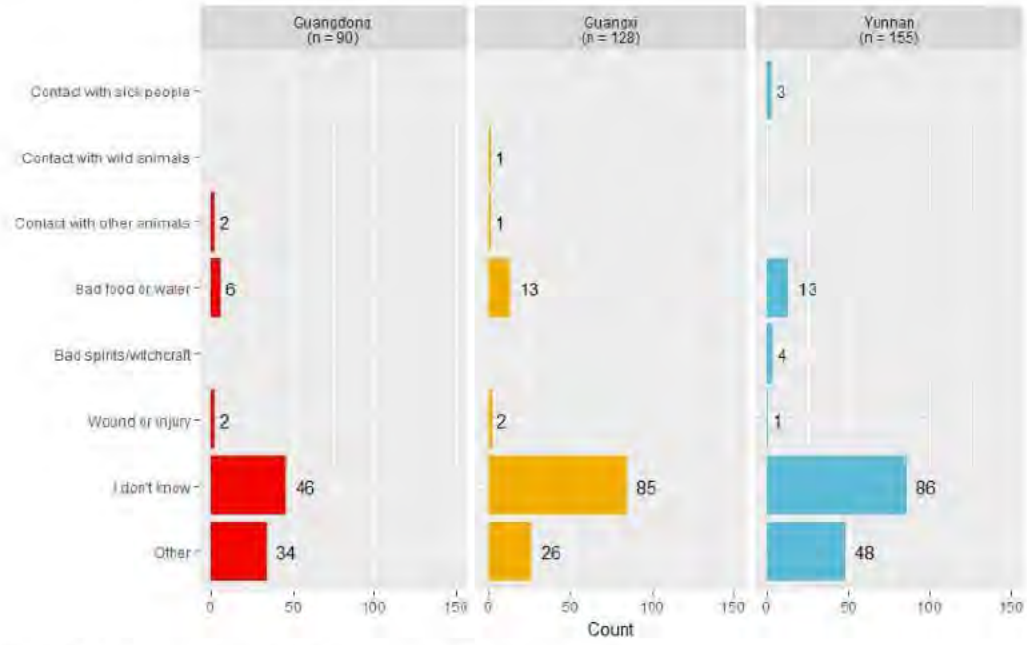
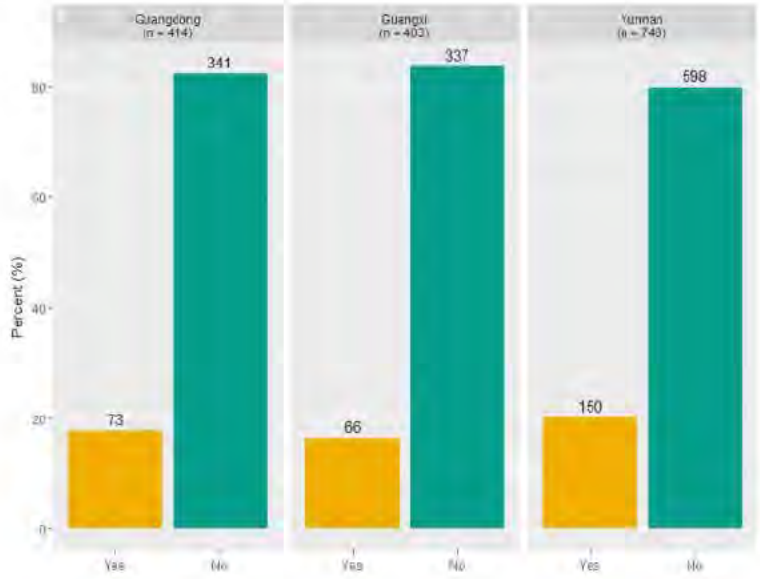


Figure 12: *Reported cause of sickness in the past year.*

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)**.

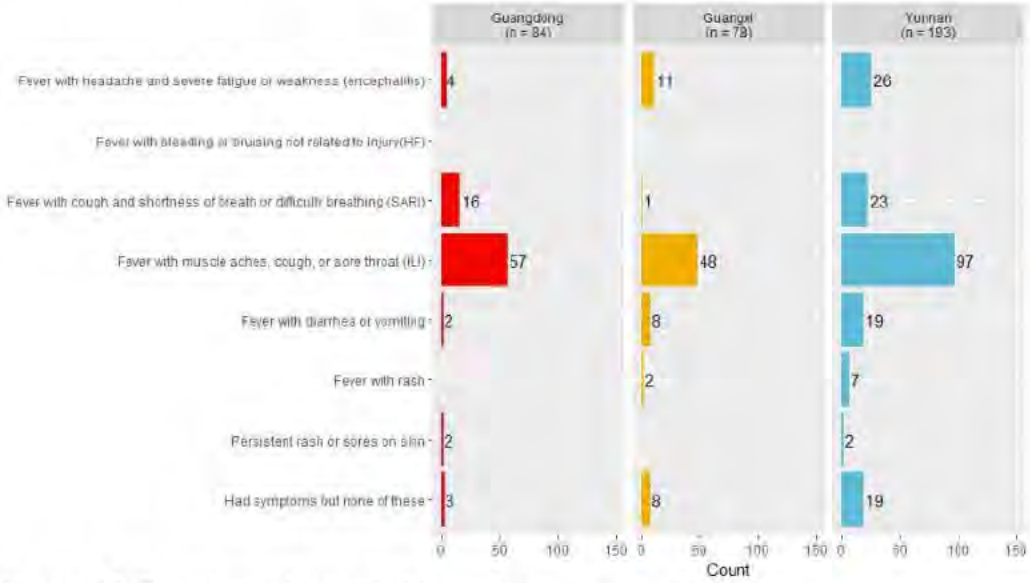


Figure 14: *Symptoms of household members who were ill, in past year.*

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)**.

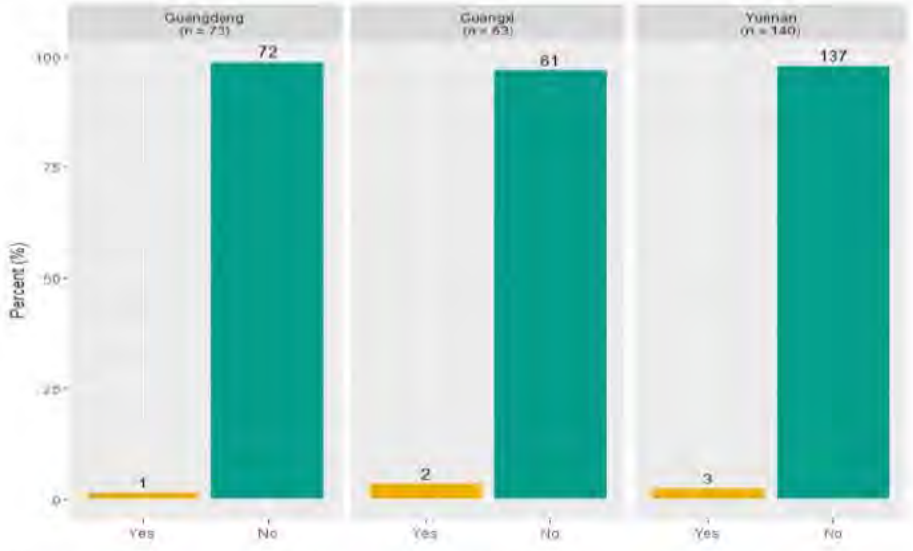


Figure 15: *Whether household members died from illness, in the past year.*

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)**.

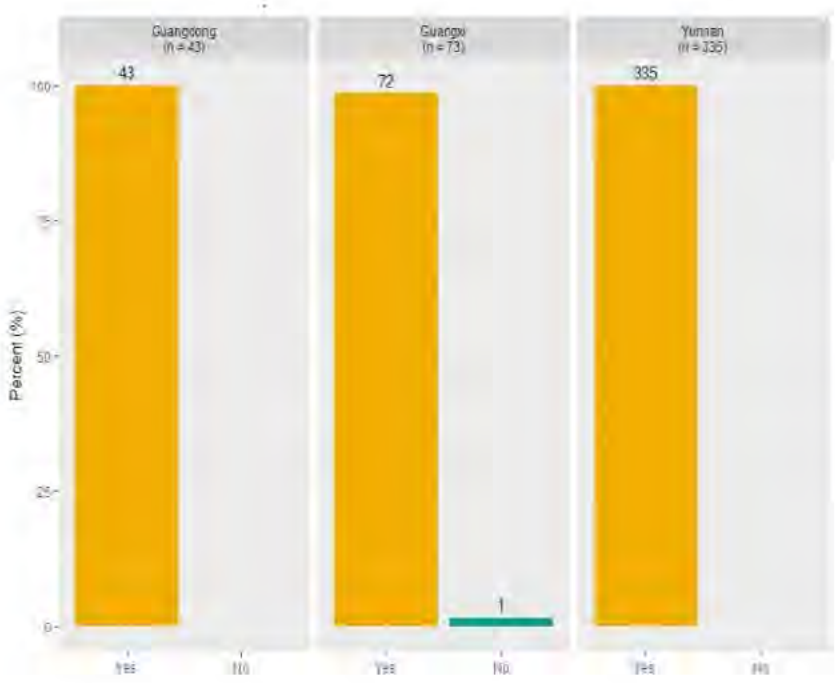
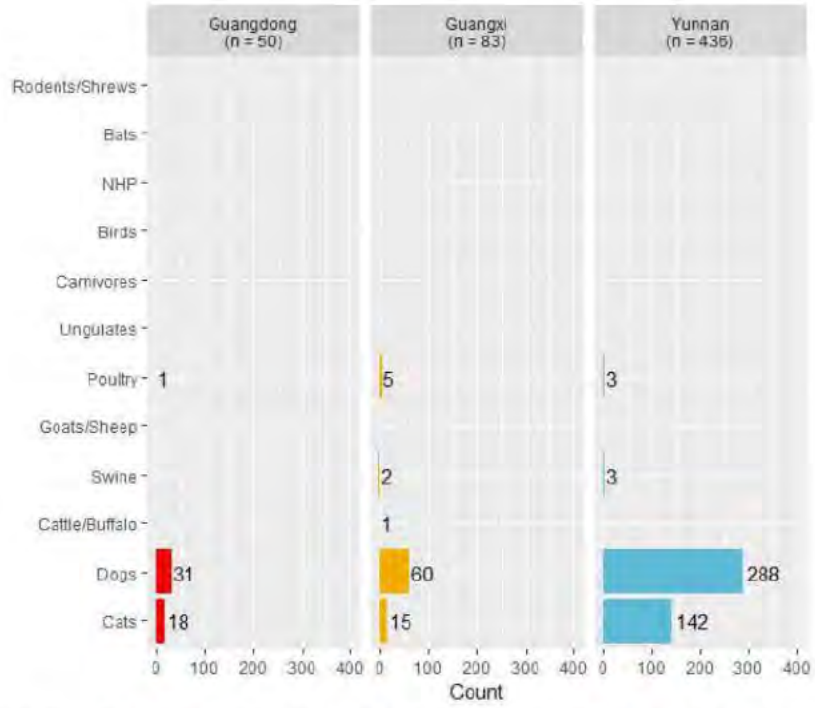


Figure 16a (top) & b (below): *(a) Whether respondents had lived with an animal as a pet, in the past year, and (b) among those who had, types of animal kept as pets.*



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)**.

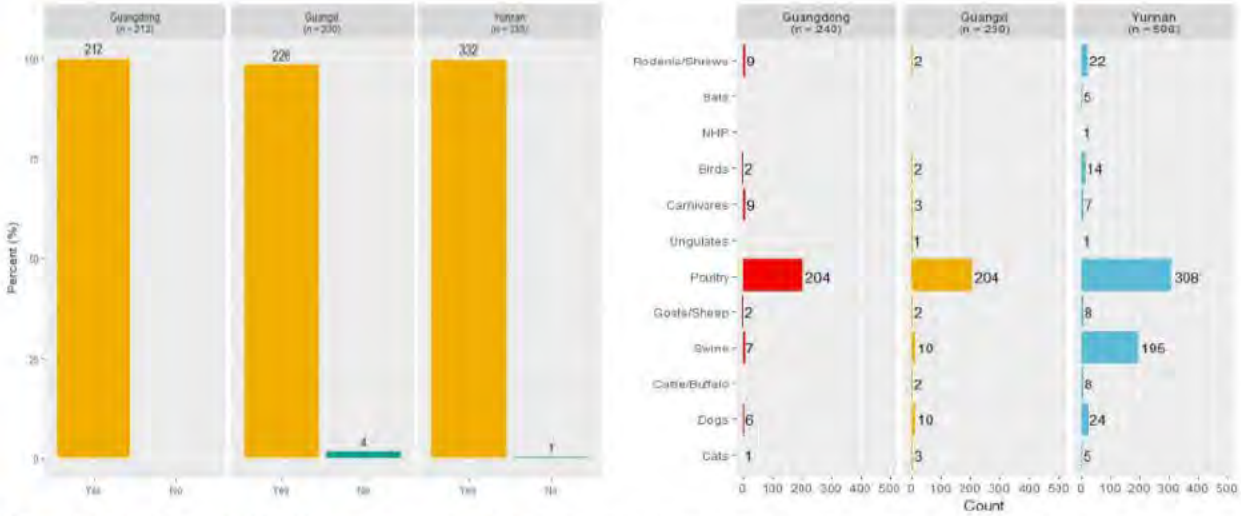
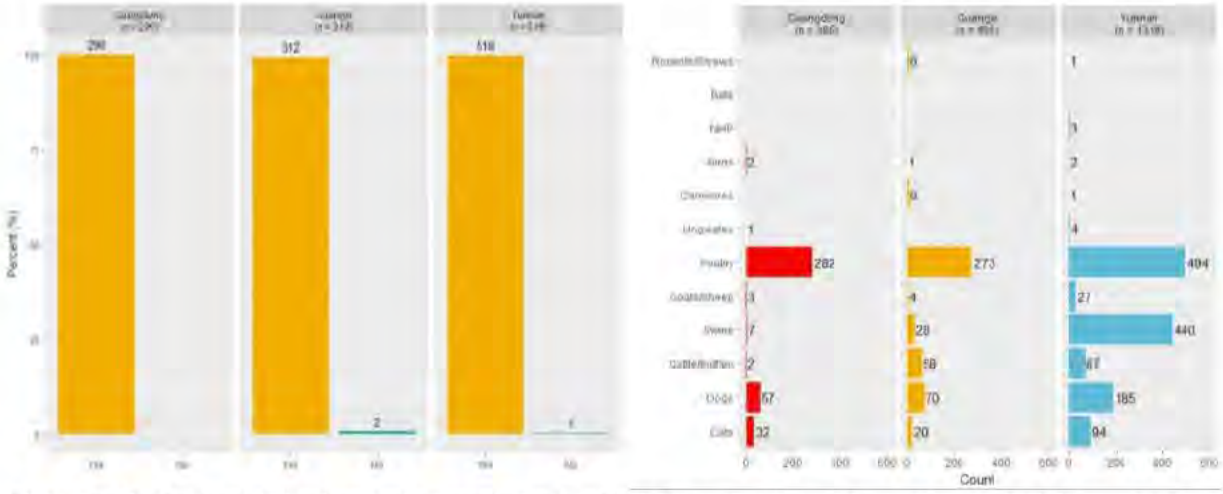


Figure 17a & b: *(a) Whether respondents had handled live animals, in the past year, and (b) among those who had, types of live animals handled.*

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)**.

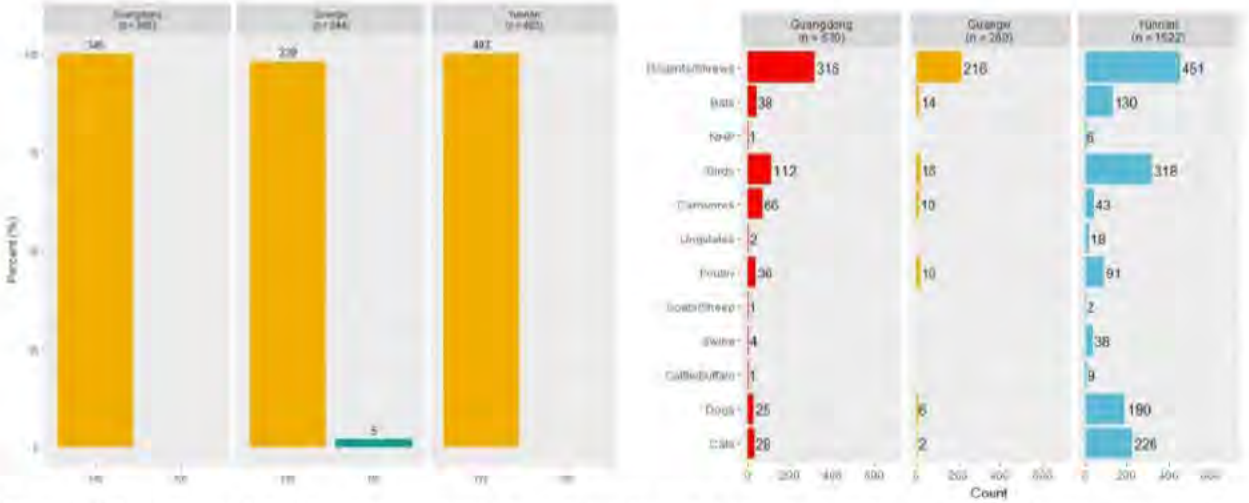


Figure 19a & b: *(a) Whether respondents had animals come inside dwelling, in the past year, and (b) among those who had, types of animals in dwelling.*

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)**.

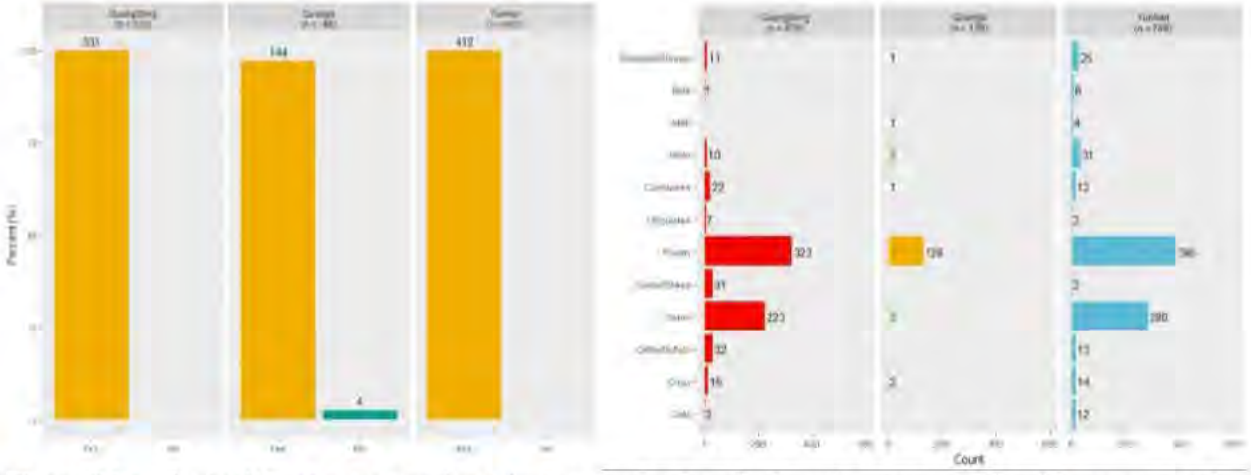


Figure 20a & b: *(a) Whether respondents had cooked or handled meat, organs or blood from a recently killed animal, in the past year, and (b) among those who had, types of animals whose meat, organs or blood was cooked or handled.*

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.

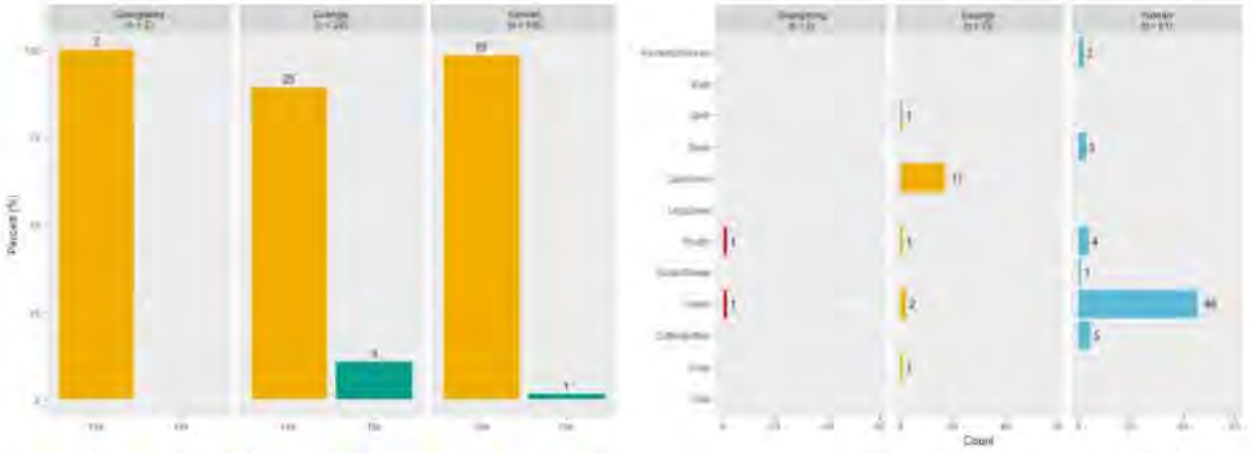


Figure 21 a & b: *(a) Whether respondents had eaten raw or undercooked meat or organs or blood, in the past year, and (b) among those who had, types of animals whose meat, organs or blood were eaten raw or undercooked.*

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

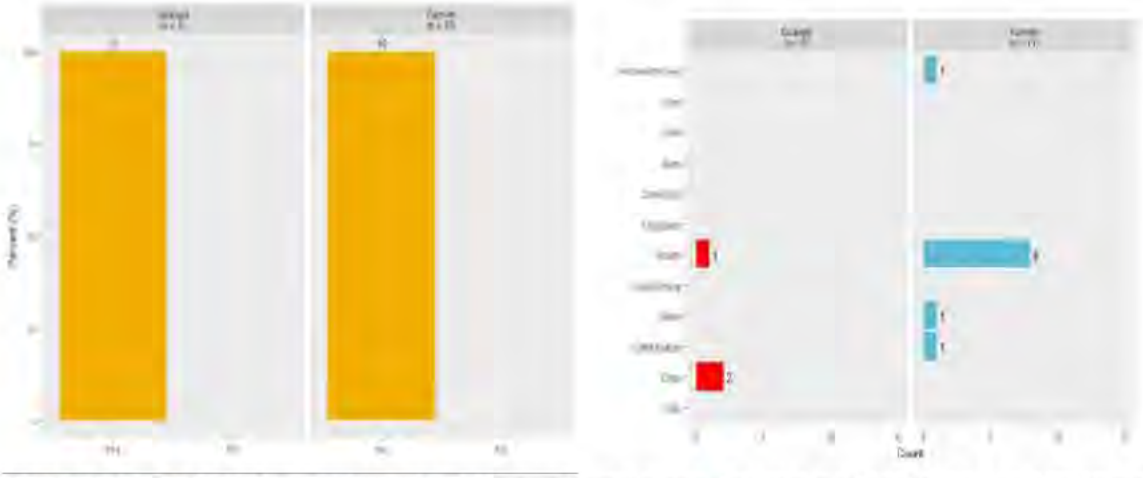


Figure 22 a & b: *(a) Whether respondents had found a dead animal and collected it to eat, share, or sell, in the past year, and (b) among those who had, types of animals that were found dead and collected to eat, share, or sell.*

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

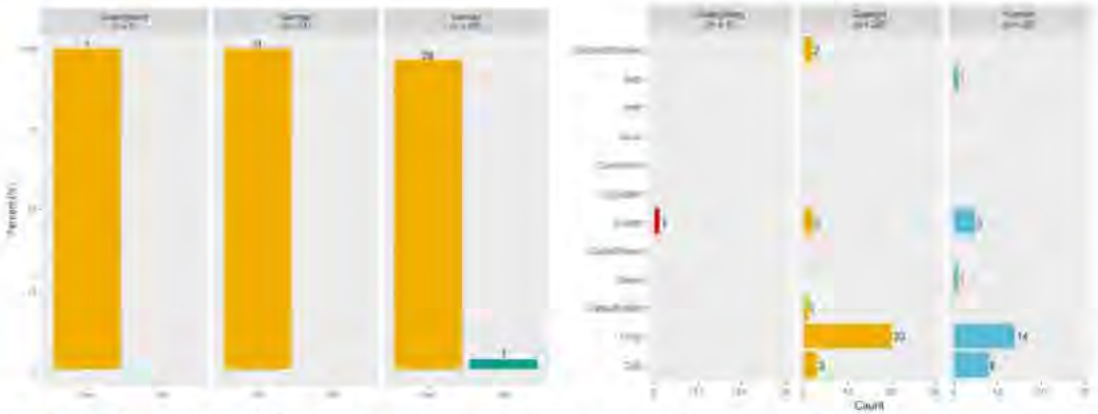


Figure 23 a & b: *(a) Whether respondents had been scratched or bitten by an animal, in the past year, and (b) among those who had, types of animals that scratched or bit respondents.*

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)**.

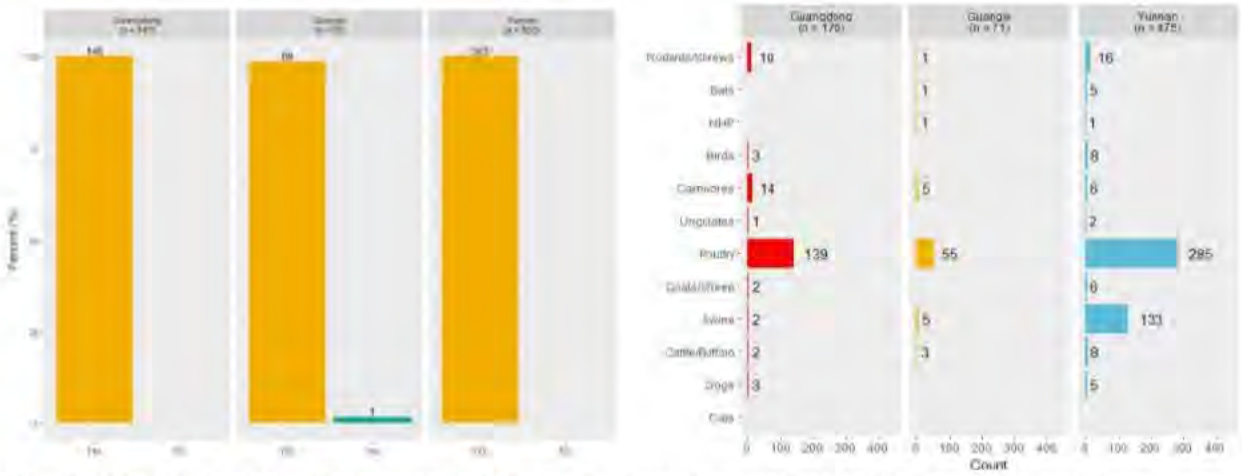


Figure 24 a & b: *(a) Whether respondents had slaughtered an animal, in the past year, and (b) among those who had, types of animals slaughtered.*

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)**.

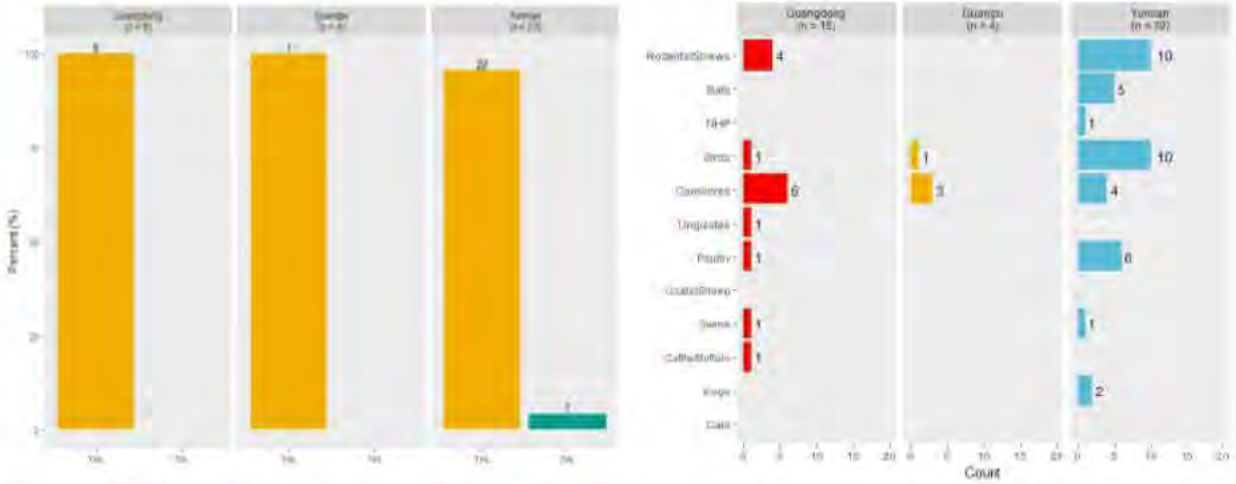


Figure 25 a & b: *(a) Whether respondents had hunted or trapped an animal, in the past year, and (b) among those who had, types of animals hunted or trapped.*

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)**.

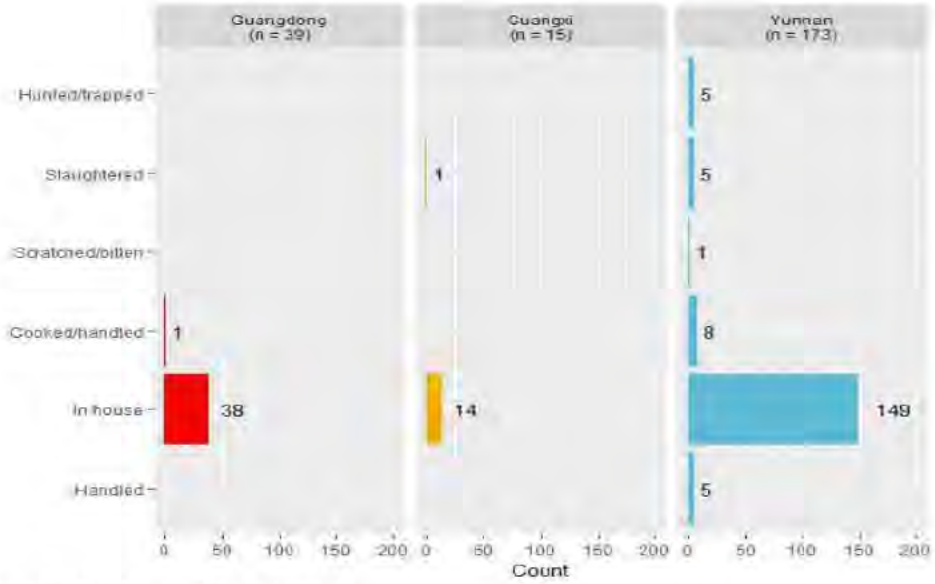


Figure 26: *Types of bat contact.*

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)**.

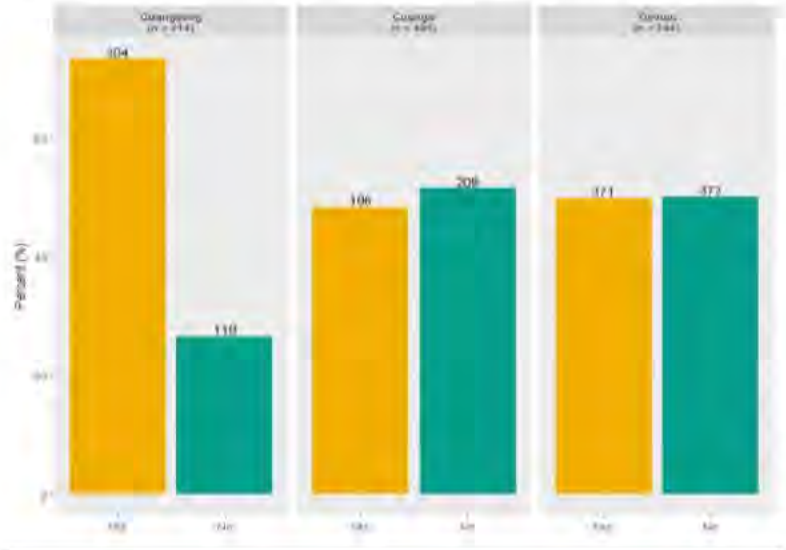


Figure 27: *Whether respondents thought that animals can spread disease.*

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)**

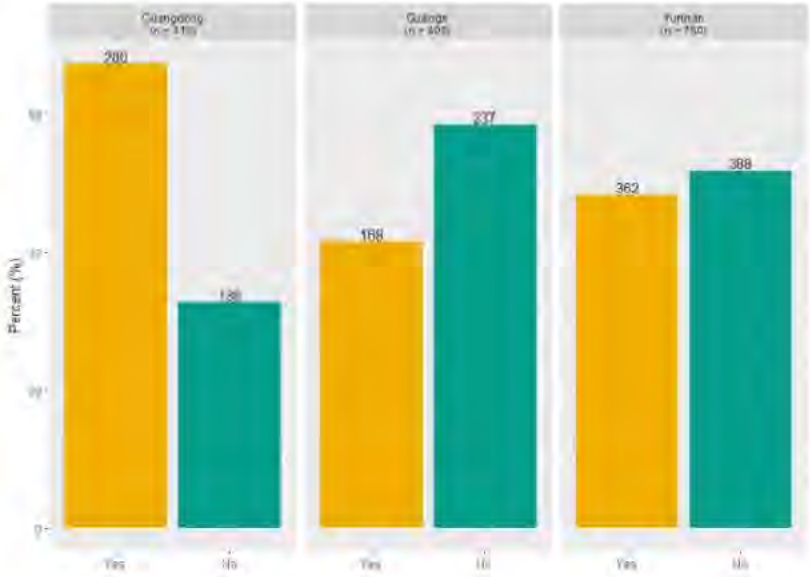


Figure 28: *Whether respondents were worried about diseases or disease outbreaks in animals at wet markets.*

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

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

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

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

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

Bat CoV PCR Detection and Sequencing from Live-Sampled Bat Populations

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

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

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

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

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

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

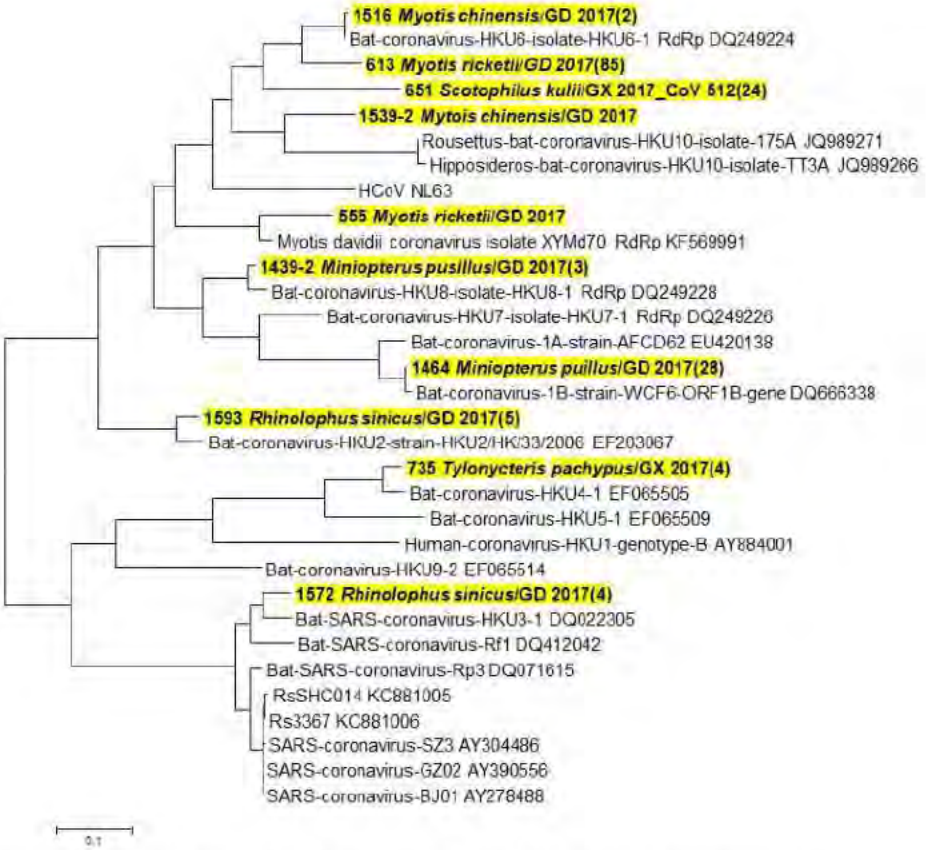


Figure 29: *Phylogenetic analysis of partial RdRp gene of CoV (440-nt partial sequence)*

Genetic Diversity and Genomic Characterization of Betacoronaviruses in Fruit Bats

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

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

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

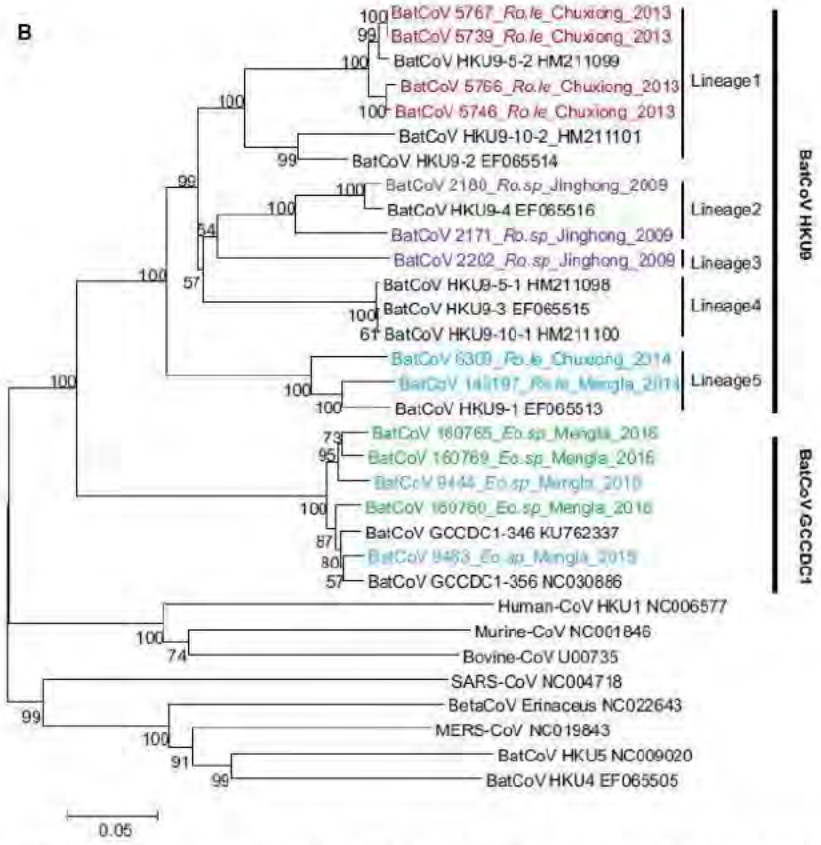


***Figure 30**. Phylogenetic analysis of full-length N gene of HKU9 and GCCDC1 CoVs*

Bat Coronavirus Host-Virus Phylogeography in China

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

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

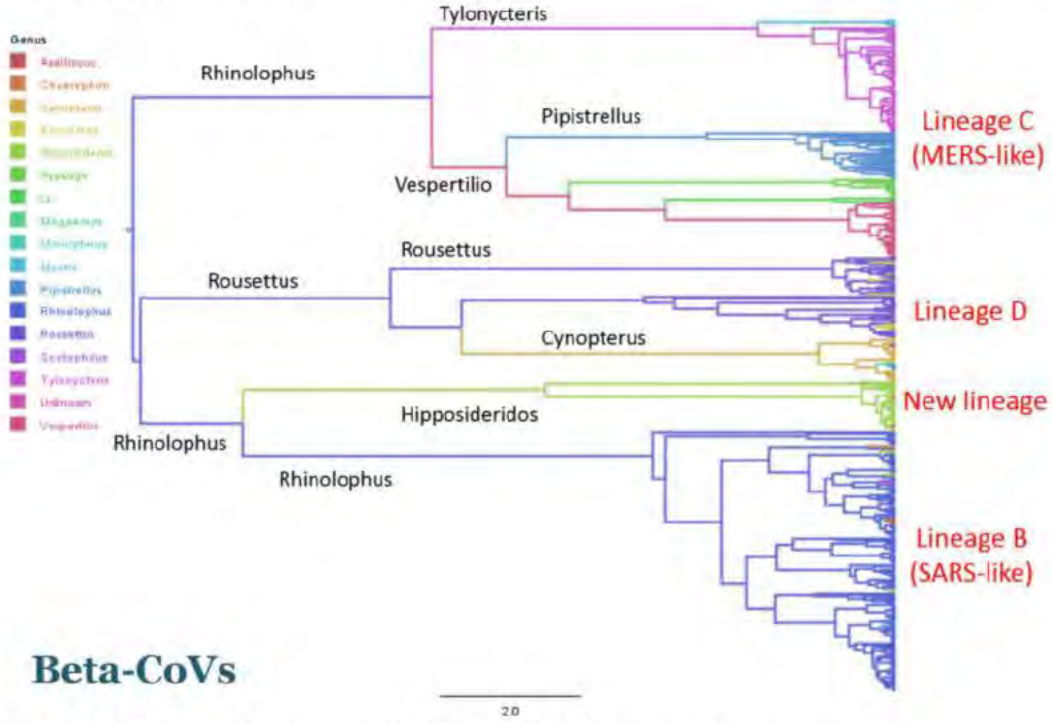


Figure 31. *Ancestral host reconstruction for Beta-CoVs, at a host genus level.*

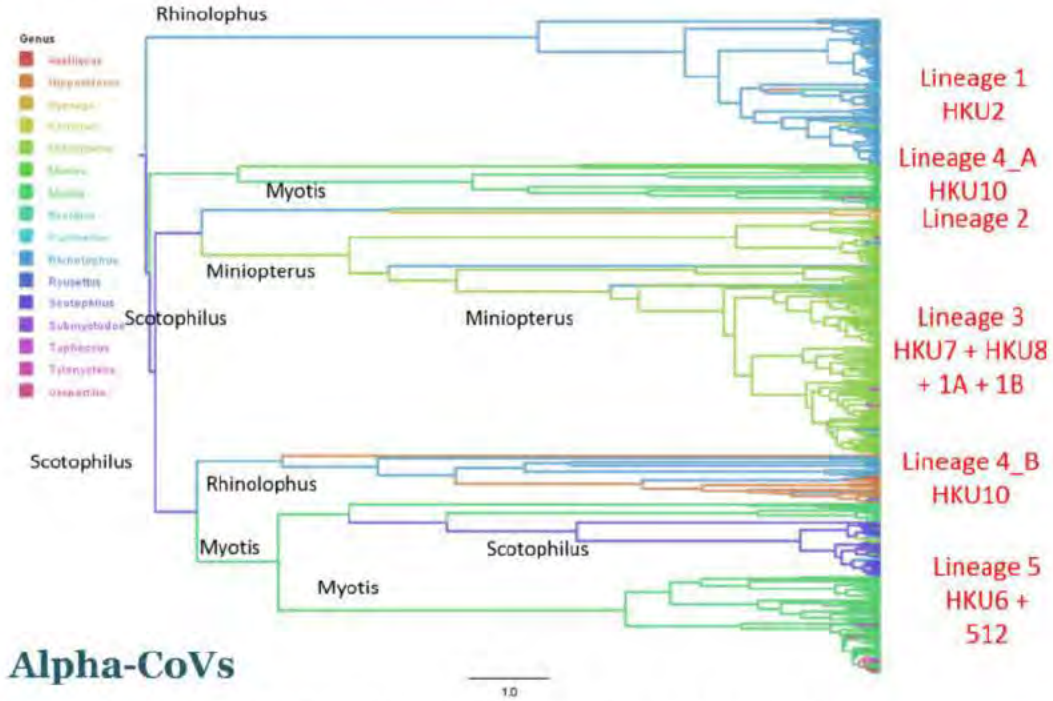


Figure 32. *Ancestral host reconstruction for Alpha-CoVs, at a host genus level.*

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.

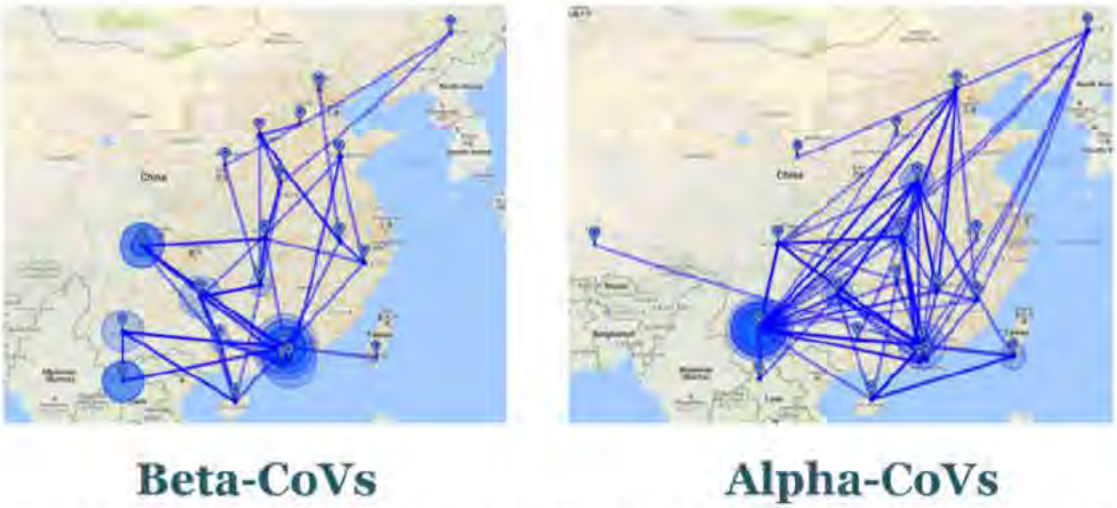


Figure 33. *Ancestral location reconstruction for Beta- and Alpha-CoVs. The bigger the circle is, the more ancestral the corresponding node is.*

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/li/GD/2013-845 and BtCoV/li/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/li/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.

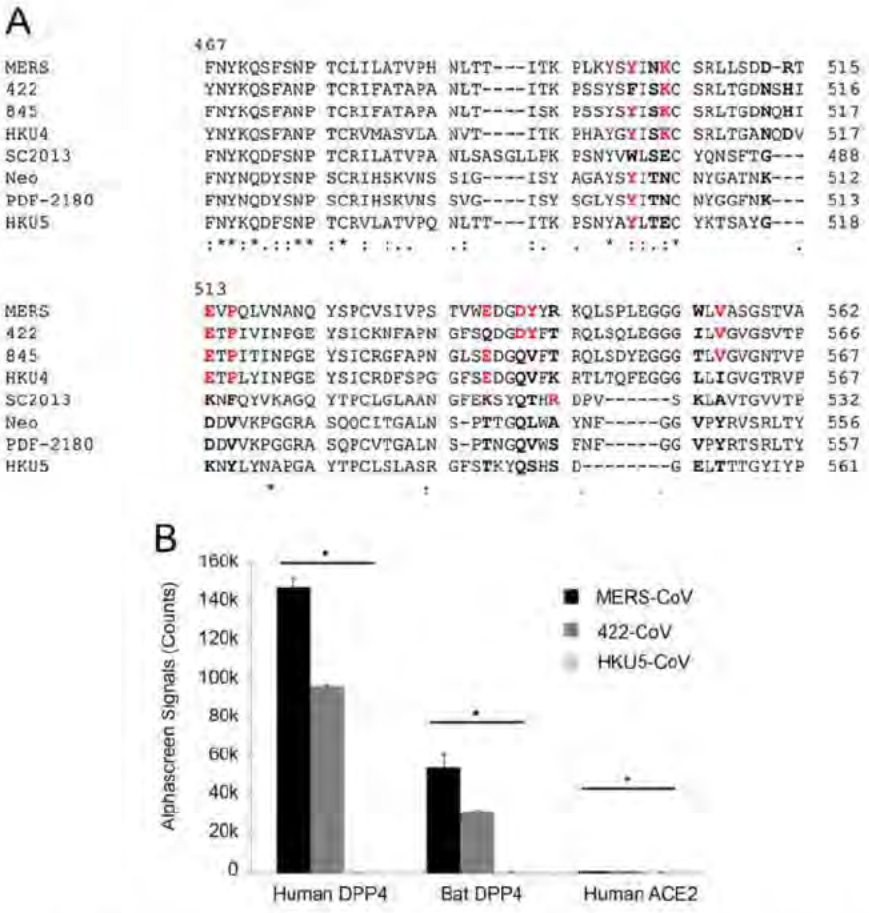


Figure 34. *BtCoV/li/GD/2014-422 RBD analysis (a) and DPP4-binding assay (b)*

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

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

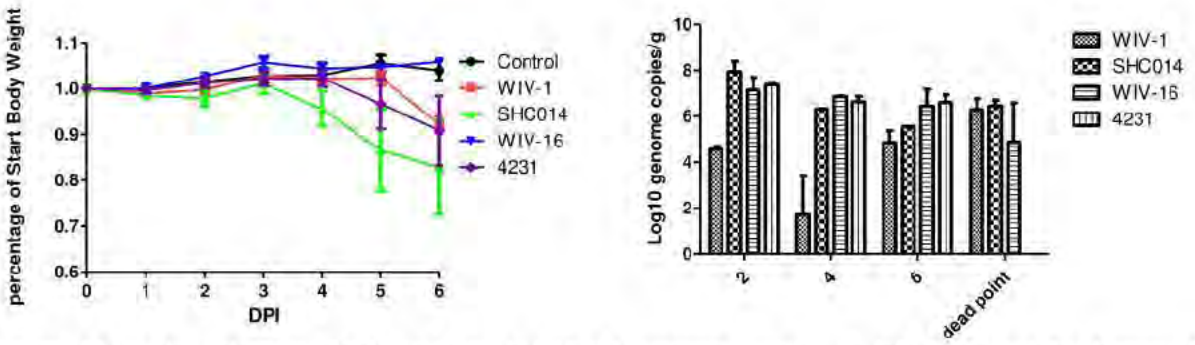


Figure 35. *In vivo infection of SARSr-CoVs in hACE2-expressing mice. (a, left) Body weight change after infection; (b, right) Viral load in lung tissues*

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.

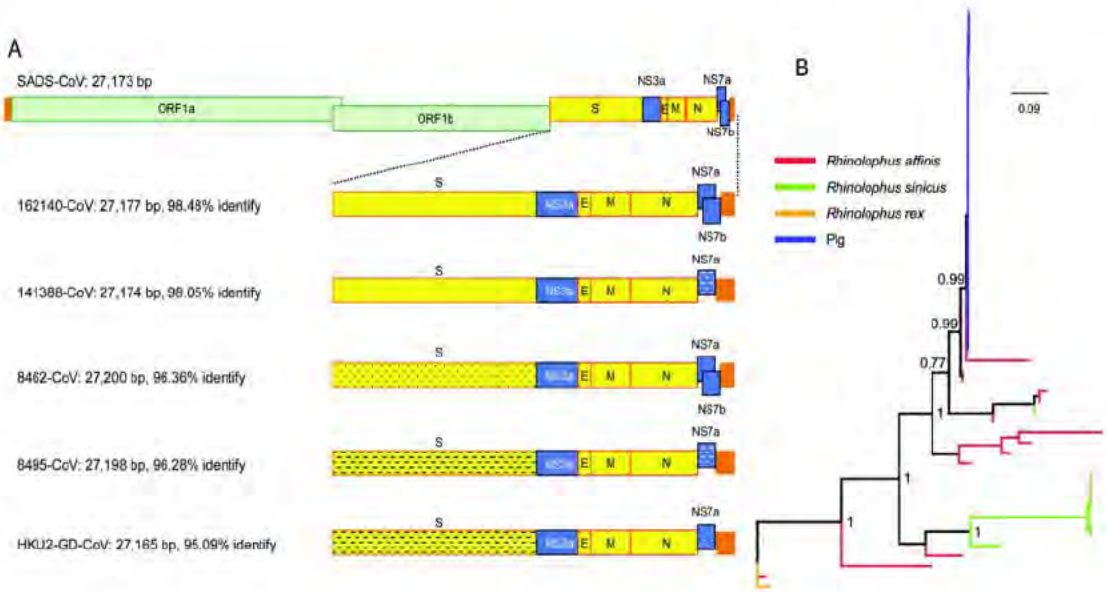
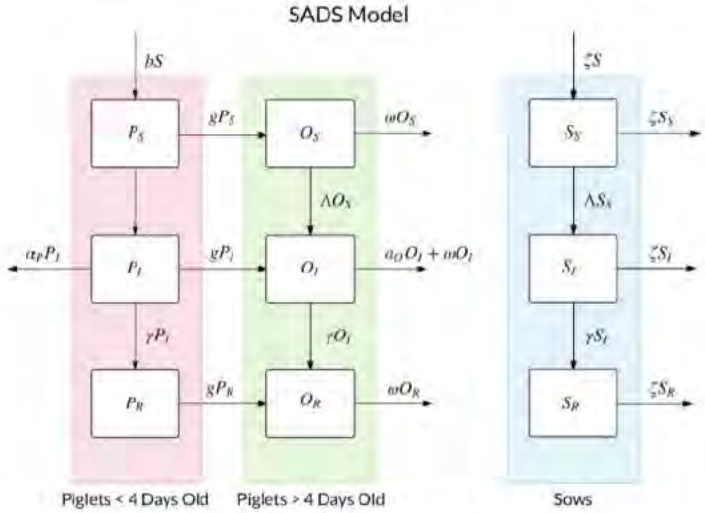


Figure 36. *Genome organization and comparison (a) and Phylogenetic analysis of S1 protein (b) of SADS-CoV and bat SADSr-CoVs*

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.



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

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

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.

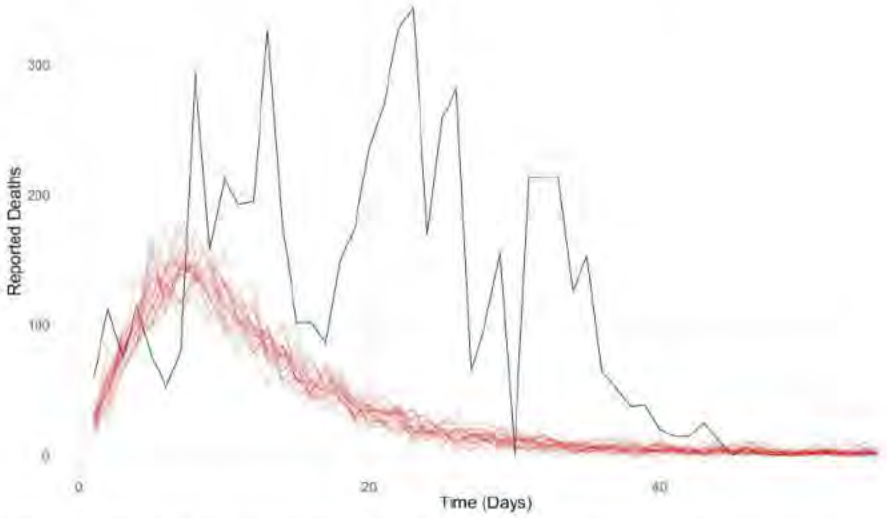


Figure 38: *Best-fit simulations (red) from an equal-mixing transmission model and actual reported death time series (black) on a SADS-infected farm.*

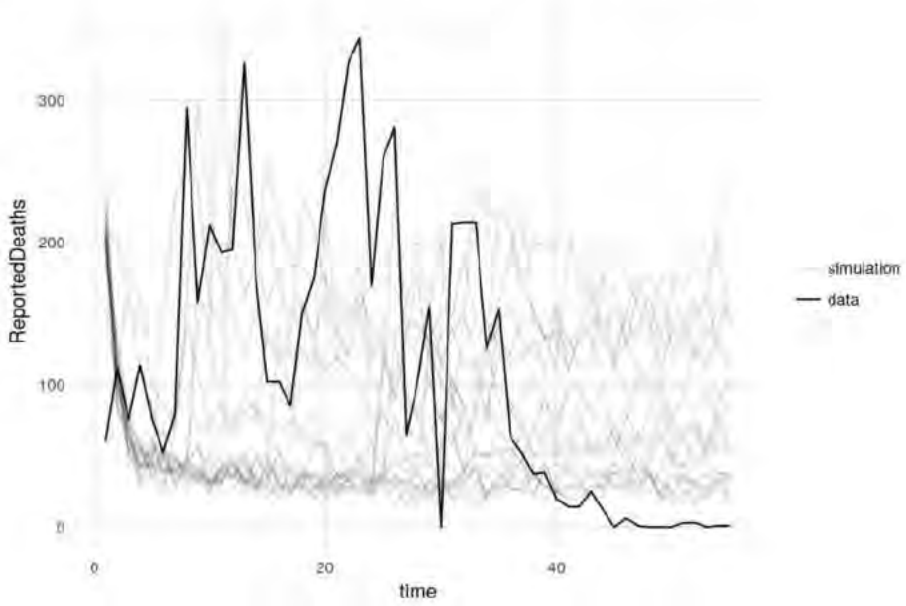


Figure 39: *Best-fit simulations (grey) from an metapopulation transmission model and actual reported death time series (black) on a SADS-infected farm.*

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

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

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

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

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

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

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

No

D.2.b New Senior/Key Personnel

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

No

D.2.c Changes in Other Support

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

No

D.2.d New Other Significant Contributors

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

No

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

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

NA

E. IMPACT

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

Not Applicable

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

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

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

Dollar Amount	Country
$201,422	CHINA

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

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

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

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

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

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

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

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

			NEW YORK NY 100012320

G.9 FOREIGN COMPONENT

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

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

G.10 ESTIMATED UNOBLIGATED BALANCE

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

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

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

No

Section 1 - Basic Information (Study 58010)

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

1.1. Study Title *

Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

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

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

1.4. Clinical Trial Questionnaire *

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

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

Section 2 - Study Population Characteristics (Study 58010)

2.1. Conditions or Focus of Study

2.2. Eligibility Criteria

2.3. Age Limits	Min Age:	Max Age:
2.4. Inclusion of Women, Minorities, and Children		
2.5. Recruitment and Retention Plan		
2.6. Recruitment Status	Not yet recruiting	
2.7. Study Timeline		

Inclusion Enrollment Reports

IER ID#	Enrollment Location Type	Enrollment Location
IER 58010	Foreign	

Inclusion Enrollment Report 58010

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

Enrollment Location Type* : ○ Domestic ● Foreign

Enrollment Country(ies): CHN: CHINA

Enrollment Location(s):

Comments:

Planned

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

Cumulative (Actual)

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	980	616	0	0	0	0	0	0	0	1596
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	0	0	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	0	0
More than One Race	0	0	0	0	0	0	0	0	0	0
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
Total	980	616	0	0	0	0	0	0	0	1596

Section 3 - Protection and Monitoring Plans (Study 58010)

3.1. Protection of Human Subjects

3.2. Is this a multi-site study that will use the same protocol to 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. Brief Summary

4.2. Study Design

4.2.a. Narrative Study Description

4.2.b. Primary Purpose

4.2.c. Interventions

Type	Name	Description

4.2.d. Study Phase

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

4.2.e. Intervention Model

4.2.f. Masking ❍ Yes ❍ No

❑ Participant ❑ Care Provider ❑ Investigator ❑ Outcomes Assessor

4.2.g. Allocation

4.3. Outcome Measures

Type	Name	Time Frame	Brief Description

4.4. Statistical Design and Power

4.5. Subject Participation Duration

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

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

4.7. Dissemination Plan