

Section 2 - Study Population Characteristics (Study 1)

2.1. Conditions or Focus of Study

- Emerging zoonotic disease from bat Coronavirus

2.2. Eligibility Criteria

Participants to be enrolled in this study will be people living, working, or visiting the high-risk sites: 1) of likely divergence of SARSr-CoVs; 2) with a high diversity of SARSr-CoVs within the S protein sequence divergence of 5-25%; and 3) with high rates of human-wildlife interactions in four provinces of Yunnan, Guangxi, Guizhou, and Guangdong in China, who meet the inclusion criteria outlined below. Study sites are prioritized according to ecological and epidemiological conditions associated with a high risk for SARSr-CoVs spillover.

Research participants will be enrolled in two settings of community and hospital or clinic.

Community

People living, working, or visiting targeted high-risk communities (as defined above) who have close contact with bats, we anticipate interviewing and collecting biological samples from individuals with a range of exposure to bats. Enrolled research participants will be asked to provide biological samples and complete a questionnaire that is designed to obtain information about living circumstances (e.g. distance between the living house and closest bat roost, observed bats in house), income or livelihood, experience with SARI and ILI-like illness and involvement in activities with direct or indirect (e.g. via livestock) contact with bats.

Additional inclusion criteria for community participants

- Adults (18 years of age or older) who provide informed consent
- Pregnant women will be considered eligible for inclusion

Exclusion criteria for community participants

- Adults (18 years of age or older) who are unable to provide informed consent, including individuals with physiologically or medically induced cognitive impairments
- Individuals under 18 years of age
- Prisoners

Hospital or clinic

Patients at clinics or hospitals presenting with clinically defined symptoms of severe/acute respiratory illness (SARI/ARI) and/or influenza-like illness (ILI) with unknown origin. As with the community-based group, biological samples will be collected from the patients, and the patients or his/her designate will complete a questionnaire. We will follow up with these participants 35 days after enrollment to collect another biological sample to assess the development of IgG and collect additional data on the course of symptoms in the interim period.

Additional inclusion criteria for hospital or clinic participants

- Adults (18 years of age or greater) who provide informed consent
- Children aged 12 years or older with an accompanying parent or guardian who is able to provide informed consent, with the assent of children 12 years or older also required
- Pregnant women will be considered eligible for inclusion

Exclusion criteria for hospital or clinic participants

- Individuals over the age of 12 years who refuse to provide informed consent
- Adults unable to provide informed consent, including individuals with physiologically or medically induced cognitive impairments
- Children, aged 12-17, without an accompanying parent or guardian who is able to provide informed consent, or a child aged 12 to 17 who is unable or unwilling to provide assent
- Children < 12 years of age
- Prisoners

2.3. Age Limits Min Age: 12 Years Max Age: N/A (No limit)

2.4. Inclusion of Women, Minorities, and Children NIAID_COV_2019_Inclusion_of_Women_Minorities_Children_Final.pdf

2.5. Recruitment and Retention Plan NIAID_COV_2019_Recruitment_Retention_Final.pdf

2.6. Recruitment Status Not yet recruiting

2.7. Study Timeline NIAID_COV_2019_Study_Timeline_Final.pdf

2.8. Enrollment of First Subject 06/01/2020 Anticipated

INCLUSION OF WOMEN AND MINORITIES:

This proposal will enroll men and women as study participants without regard to ethnicity.

- At community sites, exposure to bats in working and living environments will be the primary criteria for identifying participants in community. We will make every effort to have men and women equally represented in this study and no individuals will be excluded based on ethnicity.
- At clinic sites, only patients who present at the health facility who meet the clinical case definition of 1) severe/acute respiratory illness (SARI/ARI) of unknown origin; or 2) Influenza-like illness (ILI) of unknown origin will be recruited for this study, and no patients will be excluded (or included) based on ethnicity or gender.

INCLUSION OF CHILDREN:

Children aged 12 years or older will be included in the clinical syndromic study.

- Previous clinic-based studies have shown that children are one of the major populations who are affected by the severe/acute respiratory illness (SARI/ARI) and/or Influenza-like illness (ILI).
- Children aged 12 years or older are post-primary school and are able to respond to the questionnaire on their own which increases the reliability of responses.
- Our human research team at the Institute of Pathogen Biology are all well-trained and have extensive experience working with children at this age, as well as their parents, in a clinical setting since 2009.
- Every effort will be made to protect the privacy, dignity, and well-being of children who participate in this study.
- Inclusion of children in the study would increase the sample size to allow for the estimation of effect sizes of behavioral risk factors by two-fold (2X) or greater with 80% power.

We will not include children in the community-based surveillance because children in target communities are mainly school children who have very limited exposures to bats or other wild animals under the scenarios of interest to the study, prolonged time spent in the forests or markets.

RECRUITMENT AND RETENTION PLAN

In order to improve recruitment within target communities, introductory visits will be made by project staff to each of the selected sites. These visits will be advertised through word of mouth and a project description letter to town/city leaders that can be posted in a central community location. The letter will inform the community that a team will be coming on a particular day(s) to discuss health issues related to animal contact. The letter will not be advertised for recruitment purposes. It will only be used to inform the community of the research visits. The project description letter will be written in the local language with a Flesch–Kincaid readability score equivalent to a 7th grade and up level (post-primary in China), to assure that potential community participants understand the study purpose, eligibility, and inclusion guidelines.

During community visits, discussions and meetings will be held firstly with local authorities and community leaders to introduce our project, and when appropriate and following approval from local authorities, the study team will post flyers to inform the community when the team will be coming back to speak about enrollment. This “town hall” style meeting will be completely voluntary, and, based on our experience, those interested would likely attend. Although local authorities may be present to introduce the study team members, they will not be involved in the recruitment and/or consent of the participants for the study. If research visits or recruitment are held at a workplace, subjects will be clearly informed during this recruitment process that their participation in the study is voluntary and will not impact their employment, nor will information discussed be shared with employers. With the local permission and accompanied will local authorities or community leaders, study team members will also engage in community ‘walkabouts’ during which they will discuss study details, as well as dates, times, and locations for enrollment and participation in the study.

Participation in the study will be strictly voluntary and will require signed informed consent for all participants and signed assent for clinical participants aged 12-17 along with parent or guardian consent. Participants will be given a consent form prior to being asked to participate in this study. Our research staff will read the consent form to potential participants, and they will review the consent form with the research staff and be given time to ask questions. After reviewing the consent form, study staff will explain details of the study including: why they were selected, what the study procedures are and what will be expected from them, potential risks and benefits of their participation, that their participation is completely voluntary, and that they can withdraw their participation at any time. Responses will be kept strictly confidential. Measures will be taken to assure the privacy, dignity, and respect of each participant. During training of research staff, we will emphasize the importance of avoiding coercion and protecting the privacy of participants.

Community-based recruitment: Participants from the community will be recruited through town hall meetings and community ‘walkabouts’ as described above. Meeting dates, times, and locations for enrollment and participation will be shared during these activities, and participants who wish to enroll can volunteer to participate at these times and locations.

Clinic-based recruitment: Patients eligible for enrollment will be identified at intake areas or in the emergency room, ward, or intensive care unit of each participating clinic and hospital by clinic staff, according to standard operating procedures at collaborating sites. Employed staff at each location will identify potential participants meeting the clinical case definition of severe/acute respiratory illness (SARI/ARI) and/or influenza-like illness (ILI) with unknown origin. Patients will be screened for eligibility according to the inclusion/exclusion criteria based on available clinical information. For larger provincial-level hospitals, interval sampling will be implemented by selecting every Nth case at the site among those individuals who meet enrollment criteria. The interval will be determined by local implementing partners based on an evaluation of the expected number of cases presenting at the site within a given year in order to best meet study design and sample size criteria. In terms of retention, we will express our gratitude to subjects for their participation and discuss the importance of the follow-up data collection. Nonetheless, we expect to have an approximate 40% loss to follow up and have included this in our sample size calculations.

STUDY TIMELINE

Patients/participants will be asked to volunteer approximately 1 hour of their time for participation in the study, including providing biological samples and completing the questionnaire at each sampling time point.

This will be an ongoing, five-year project (June 01, 2019 -- May 31, 2024). We anticipate to starting human subject enrollment on June 01, 2020, and completion of preliminary analyses is expected in 2024.

Inclusion Enrollment Reports

IER ID#	Enrollment Location Type	Enrollment Location
<u>Study 1, IER 1</u>	Foreign	Local community and hospital/clinic in Yunnan, Guangdong, Guangxi, Guizhou Provinces

Inclusion Enrollment Report 1

Using an Existing Dataset or Resource* : Yes No

Enrollment Location Type* : Domestic Foreign

Enrollment Country(ies): CHN: CHINA

Enrollment Location(s): Local community and hospital/clinic in Yunnan, Guangdong, Guangxi, Guizhou Provinces

Comments: This is a renewal, the cumulative enrollment from the previous funding period 5R01AI110964-05 is 980 females and 616 males, in total 1,596 Asians.
We don't plan to use the existing dataset.

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	4675	4675	0	0	9350
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	4675	4675	0	0	9350

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	0	0	0	0	0	0	0	0	0	0
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	0	0	0	0	0	0	0	0	0	0

Section 3 - Protection and Monitoring Plans (Study 1)

3.1. Protection of Human Subjects

NIAID_COV_2019_Protection_Human_Subjects_Final.pdf

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

PROTECTION OF HUMAN SUBJECTS:

1. Risks to Human Subjects

1.1 Human Subjects Involvement, Characteristics, and Design

This project is a study of human exposure to animal coronaviruses in southern China. Subjects will be enrolled on a voluntary basis and informed consent will be obtained from all participants. Consenting participants will provide biological samples and complete a questionnaire. Subjects will be individuals: 1) who are highly exposed to bats in community settings, including through hunting, butchering, or general handling within the context of their living or working environment (≥ 18 years old); and 2) patients admitted to hospitals and clinics presenting with disease symptoms of clinically-defined severe/acute respiratory illness (SARI/ARI) or Influenza-like illness (ILI) of unknown origin (≥ 12 years old).

The study population will be selected from the Yunnan, Guangxi, Guangdong, and Guizhou provinces of China. We will aim to enroll: 1) in 12 clinic sites across the four provinces, 2,750 individuals (accounting for an estimated 40% loss from follow-up); and 2) in 8 community sites, 1,650 individuals per each of the four provinces, pooled across two sites for each province for a total of 6,660 ($1,650 \times 4$) participants, allowing us to make province-level comparisons of differing effects (one time data collection, no follow-up among community participants). The community and clinical sites are further defined in “**Specific Aim 2: Using community-based and clinical biological-behavioral surveys to identify SARSr-CoV spillover, routes of exposure and public health consequences of human infection**”.

There are no data to suggest a gender or ethnic bias for coronavirus exposure or infection, therefore subjects will be enrolled based on exposure criteria, and subjects will not be excluded based on ethnicity or gender. We will also stratify sampling to ensure representation of sex, demographic, and socio-economic factors in each community site.

1.2 Sources of Materials

Samples to be collected and screened for coronaviruses include whole blood and nasal/oropharyngeal swabs. Samples will be collected and a questionnaire will be administered by trained medical personnel from the local CDC, hospitals, and clinics. In community sites, whole blood samples (only) will be collected once during Years 2-4 of the study, and samples will be screened for coronaviruses using developed ELISA at the Institute of Pathogen Biology and the Wuhan Institute of Virology. In clinic sites, both whole blood samples and nasal/oropharyngeal swabs will be collected at enrollment, and samples will be screened for coronaviruses using ELISA and consensus PCR (cPCR). Patients who test positive for coronavirus or antibodies to coronavirus will be followed up 35 days after enrollment, when additional blood samples will be collected for serological testing with ELISA.

At the enrollment, a standardized questionnaire will be administered at both community and clinic sites to collect data on living circumstances (e.g. distance between the living house and closest bat roost, observed bats in house), income or livelihood, experience with SARI and ILI-like illness and involvement in activities with direct or indirect (e.g. via livestock) contact with bats. During the follow-up with clinic study participants, a standardized questionnaire supplement will be administered to collect additional data on the course of symptoms in the interim period. All electronic data will be password protected, and all hardcopy files and biological samples will be stored in secure storage facilities. All consent forms will be stored separately from any data in separated locked filing cabinets.

1.3 Potential Risks

The potential risks to study participants resulting from study participation are minimal. The volume of blood being collected is within normal safety limits. The questionnaire will be designed to assess exposure risk, and may ask personal questions, but they will be conducted in private and confidentially to protect privacy. There may be some stress to subjects who are informed that they have been exposed to an animal virus, but counseling will be available and options for medical care will be included in the discussion.

2. Adequacy of Protection against Risks

2.1 Recruitment and Informed Consent

Potential study participants at each site will be identified by well-trained in-country research team in partnership with local CDC staff (for community participants) and medical personnel (for clinic participants). The team will be thoroughly trained on communicating the research objectives, what is being asked of participants, any risks or benefits, and will be able to address any questions that potential subjects may have. Both written and oral descriptions of the study details will be provided in Chinese Mandarin (or orally via an interpreter in local dialects if necessary) as part of the informed consent process. Contact details of the collaborators at the local CDC or hospital and the study PI will be provided to all subjects, and CDC or hospital personnel on the research team will be available onsite to answer questions from the study subjects. Test results will be communicated to each subject and counseling offered to minimize stress.

2.2 Protection against Risks

After the informed consent process, the questionnaire will be conducted in private, ensuring that others cannot overhear responses. Individual sessions will be held in areas where there are no other individuals within a 10-foot distance. A barrier will be created so that no other individuals can view the participants during their interview. Depending on the location, this could be a private room, behind a building or fence, or behind a line of trees, obstructing view so that confidentiality may be maintained. The interview team will take care to pair interviewers and respondents by sex to the best of their ability to increase the level of comfort of the participant and the team will ensure the privacy and confidentiality of responses. Children will not be interviewed in the absence of a parent or guardian. This study will not involve greater than minimal risk, and every effort will be made to ensure the privacy, dignity, and well-being of children who participate in this study.

3. Potential Benefits to Subjects and Others

There are potential benefits to the study subjects including receiving a physical exam/health check from a medical officer and the potential benefit of identifying a health hazard. At the conclusion of the study, we will deliver an educational workshop for high risk individuals (open to study subjects and non-study subjects) describing the health benefits of using PPE and hand-washing during animal handling activities throughout the day, as well as to share other prevention interventions that emerge from the research data.

4. The Importance of Knowledge to be Gained

There are valuable potential benefits to the general public from the knowledge to be gained by this study, as it may identify sources of zoonotic coronaviruses in the market system or through hunting. Avoidance of these animals or extra care when handling them may substantially reduce the risk of CoVs (and other zoonotic pathogen) transmission.

Section 4 - Protocol Synopsis (Study 1)

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

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
The form does not have any delayed onset studies			

VERTEBRATE ANIMALS:

1. Detailed description of animal use.

Work with vertebrate animals will be conducted at Wuhan University at the School of Medicine in Wuhan, China and the University of North Carolina, Chapel Hill, USA.

Capture and sampling techniques for all wild animals (bats) described in this study have been previously approved by multiple Institutional Animal Care and Use Committees (IACUCs) for projects led by EcoHealth Alliance. These institutions include: UC Davis IACUC (Mazet and Epstein; UC Davis 15898; current); and The Cummings School of Veterinary Medicine at Tufts University (Olival and Epstein, current), Animal Welfare Assurance (#A4059-01) on file with the Office of Laboratory Animal Welfare at the National Institutes of Health. We have prepared a draft IACUC application for this project, and will submit it within 1 month of the project's start date (to Tufts University) to minimize delays in beginning Year 1 field sampling.

Experimental work using humanized mice will be conducted at the Center for Animal Experiment Biosafety 3 lab of Wuhan University at the School of Medicine in Wuhan, China and the University of North Carolina at Chapel Hill, the Institute for Pathogen Biology. The Wuhan laboratory is AAALAC accredited and has both an Institutional Biosafety Committee and an Institutional Animal Care and Use Committee. We will submit our protocols for IACUC approval should this proposal be funded. Conditions for animal use are described below. Both laboratories (Wuhan and UNC) have Internal Biosafety Committees and are accredited BSL-2 and BSL 3 laboratories. Animals will be housed in a BSL-3 facility and will be under the care of a full-time veterinarian. All experimental work using infectious material will be conducted under appropriate biosafety standards. Disposal of hazardous materials will be conducted according to the institutional biosafety regulations.

Note: The majority of wild bats captured and sampled will be done using non-destructive, techniques. In a small number of instances (~ 2 bats per species for previously unsampled *Rhinolophus* sp.), where intestine and lung tissue is required to establish cell lines, animals will be humanely euthanized and a necropsy performed according to accepted protocols (see euthanasia section)

Bat capture. Free-ranging bats will be captured using either a mist net or harp trap. The net system is manned by two people during the entire capture period, and bats are removed from the net as soon as they become entangled to minimize stress and prevent injury. In the Co-PI's (Dr. Olival) experience, a maximum of 20-30 bats can be safely held and processed by a team of three people per trapping period. Duration of trapping will depend on the capture rate. Bats are placed into a pillowcase or small cloth bag and hung from a branch or post until samples are collected. Bats are held for a maximum of six hours (typically less than 3 hours), and released after sampling.

Laboratory mice. Lab mice will be sourced commercially by the Wuhan Center for Animal Experiment at Wuhan University.

Sample Collection:

Bats: Bats will be manually restrained during sampling. Depending on the species and size of bat, swabs will be taken from the oropharynx, urogenital tract, and rectum. Fresh feces will be collected if available, in which case a rectal swab will not be collected. Blood will be collected from fruit bats either from the cephalic vein or from the radial artery or vein using a 25 gauge needle and 1cc syringe. Blood

will be collected from bats weighing less than 100g according to published techniques (126).

Rodents: Anesthesia for captive small rodents will be conducted using plastic tubes, with the animals transferred directly from the traps to the tubes containing a cotton swab soaked in ether, isoflurane, or methoxyflurane for anesthetic induction. For larger rodents, chemical restraint and anesthesia (ketamine alone, or ketamine combined with xylazine) will be applied either through the squeeze cages by syringe if applicable. Once anesthetized a small blood sample will be collected using a capillary tube placed into the retro-orbital sinus. Only trained technicians will perform retro-orbital bleeding and it will only be performed on anesthetized rodents. Femoral or jugular venipuncture may be used for larger rodents (e.g. rats). In all rodents, blood volumes of no more than 1% of body weight will be withdrawn. (example 0.2 ml blood from a 20 gram rodent).

Laboratory Mice. Humanized mice will be bred at the University of Wuhan and University of North Carolina at Chapel Hill. Mice will be inoculated with a specific dose (e.g. 1×10^6 TCID₅₀) of virus through different routes (intranasally and intraperitoneally). Mouse body temperature will be monitored with implanted temperature sensing microchips (LifeChip Bio-thermo, Destron Fearing), and mice will be weighed daily. Animals will be observed daily for clinical signs of illness. Moribund mice will be euthanized, according to AVMA recommendations. Live animals will be euthanized at three weeks post-inoculation and organs harvested. We will collect sera on days 10, 15 and 21 to test for neutralizing antibodies against bat CoVs. We will collect nasal washes, oral swabs, and rectal swabs, and urine every two days. These are minimally invasive procedures, and will be performed by experienced lab technicians under the supervision of a full-time veterinarian.

2. Justify use of animals, choice of species, numbers to be used. Species and number used in study: The purpose of this study is to conduct targeted, but extensive surveillance of bat populations in Southern China to detect coronaviruses that may pose a risk to the health of both humans and animals. The experimental work is designed to understand the ability of bat coronaviruses to bind to human receptors. In this renewal application, we propose a total bat sample size comparable to our initial R01 effort of ~5000 animals. These animals will be sampled from ~15-20 species collected across 4 provinces. Given ~5-12% prevalence of SARSr-CoVs in *Rhinolophus* spp. at our previous sites during our initial R01, this sample size would give us 425 (± 175) positive individual bats, and allow us to identify ~125 novel strains. Assuming a conservative prevalence rate, a sample size of $n=110$ individuals per species will allow us to detect SARSr-CoV using PCR with a power of 80%. **Wild bats:** We will sample a minimum of 110 individuals from ~15-20 different bat species from sites across four provinces in Southern China (Yunnan, Guangxi, Guizhou and Guangdong). Sampling will focus on species in the family *Rhinolophidae*, genus *Rhinolophus*., but will also include individuals in the related genera *Hipposideros* and *Aselliscus*. In every situation, sampling of wildlife will be conducted in the most humane manner while minimizing the impacts on individual animals and their wild populations. In all instances, the fewest number of animals will be sampled that will provide valid information and statistical inference for the pathogen and disease of interest and every effort will be made to minimize stress and discomfort for the animal.

A small number of bats (maximum 2 per species) representing each of the species in this study may be euthanized in order to collect lung and intestinal tissue required for characterizing coronavirus receptors. Voucher specimens may also be collected at the discretion of the team leader for the accurate identification of species using molecular methodology.

Humanized mice for experimental infection for Specific Aim 3: In order to understand whether select strains of bat-borne CoVs utilize receptors found in people have the potential to infect people, we will use Swiss albino mice (standard breed at Wuhan University) that have been genetically modified to have

human receptors. We'll infect them with cultured bat coronaviruses and determine which organs become infected and whether these mice are capable of shedding infectious virus. Humanized mice will be genetically modified to carry human ACE2 gene will be used to evaluate pathogenesis of CoVs. We cannot anticipate exactly how many viruses we will find that are candidates for experimental models, however will likely identify approximately 5-6 bat SARSr-CoV strains that will be used for mouse infection experiments. We will use 15-20 adult mice per virus strain, and therefore will require a maximum of 120 mice over the study period.

3. Provide information on veterinary care. For wild caught animals, there is no specific veterinary care that is appropriate, nor will clinical veterinary facilities be available. Animals that are injured during the capture or sampling process will be assessed by an experienced team leader, and if the animal is determined to be unlikely to survive if released, it shall be euthanized humanely (see euthanasia section). Animals will be released within hours of capture.

Laboratory mice will be housed in BSL-3 small animal facilities at the Center for Animal Experiment at Wuhan University and University of North Carolina at Chapel Hill, the Institute for Pathogen Biology. Experimental animals will be regularly monitored by experienced staff and a supervising veterinarian. The animal facility operates 24 hours a day and has full-time veterinarians on staff. All animals will be provided with food and water ad libitum and will otherwise receive standard care.

4. Procedures for ensuring animal comfort, lack of distress, pain, or injury: Wild bats will not be held longer than 6 hours during the sampling process. Co-PI Olival has extensive experience in capture, anesthesia, and sampling wildlife, especially bats. In our team's experience, bats tolerate the described procedure well. Mist nets will be attended continuously during capture periods, and bats will be extracted from the net as soon as they become entangled. This will minimize stress and injury from entanglement. Bats will be placed individually in cotton bags and hung from tree branches while awaiting processing and during recovery. The bags are sufficiently porous as to allow for ventilation and are designed for bat capture. The enclosed environment seems to calm the bats, as they do not struggle once inside, but they hang quietly – this is a standard and accepted practice in the bat research world and best way to minimize stress to the animal. Animals will be monitored by a veterinarian or experienced field team member during all stages of capture, processing, and release. Animals will be kept in a cool place while in the pillowcases.

The procedures used in this experiment (blood draw, nasal, oral, and rectal swabs) are minimally invasive. Mice that show signs of morbidity post-infection will be examined and euthanized according to AVMA standards (see below).

5. Euthanasia: In the event of injury to an animal that results in pain and suffering, and reasonable veterinary care is unavailable, the animal will be euthanized by a veterinarian or trained field team member using ketamine injected intramuscularly 37.5mg/kg and sodium pentobarbital injected intravenously at a dose of 1.0ml per 5kg injected intravenously. This protocol is in accordance with the AVMA euthanasia report (2007). Any animal that is euthanized using a chemical agent will be disposed such that it will not be permitted to enter the food supply either through markets or hunting.

SELECT AGENT RESEARCH/BIOHAZARDS. No select agent research.

Agents: SARS-related bat coronaviruses (SARSr-CoV), like WIV1, WIV16 and SHC014. These bat viruses are distantly related to the epidemic human SARS-CoV which emerged in 2003 and caused 8,000 cases and 800 deaths worldwide. While the epidemic human SARS-CoV is a BSL3 select agent, the SARSr-CoV are BSL3 pathogens in the US and not select agents. The proposal will use a SARSr-CoV molecule clone designated WIV1 during the course of these studies, which is NOT a select agent. This strain has not been shown to cause human disease or be transmissible between humans. All recombinant DNA work will use the bat SARSr-CoV WIV1 molecular clone. At the University of North Carolina (US Government select agent certified laboratory), some virus growth studies will be conducted in primary human airways, comparing wildtype SARS-CoV, WIV1 and various SARSr-CoV WIV1 chimeric virus growth kinetics. Wildtype SARS-CoV strain research will not be conducted at the Wuhan Institute of Virology.

Registration status of all entities where select agent(s) will be used. Wildtype SARS-CoV is a select agent. UNC-Chapel Hill is currently registered with the CDC for select agent use, including SARS-CoV, as required by select agent regulations (42 CFR 73). The UNC SARS select agent laboratories are routinely inspected by the environmental health and safety department at UNC and by the CDC. Workers receive select agent and BSL3 training focused on SARS-CoV safety, procedures and protective clothing/PAPR training each year.

Introduction and Background. SARS-CoV caused outbreaks with significant case fatality rates, and there are no vaccines available for this agent. SARS-CoV is classified as a BSL-3 select agent. Wildtype SARS-CoV is currently thought extinct in the wild. The work proposed in this application will involve two aspects: field work and laboratory work, focusing on distantly SARS-like bat coronaviruses (SARSr-CoV). Fieldwork involves the highest risk of exposure to SARSr-related or other bat CoVs, while working in caves with high bat density overhead and the potential for fecal dust to be inhaled. There is also some risk of exposure to pathogens or physical injury while handling bats, civets, rodents or other animals, their blood samples or their excreta. The Co-PIs and field team have extensive experience and certification working with wildlife species and high-biosecurity pathogens (Nipah virus, ebolavirus, SARS), and great care will be taken in the field to limit the risk of accidental exposure to known or unknown animal pathogens. We have strict procedures for handling bats and working with samples from them as they are secured in the field and transported to the lab. Field team members handling animals will be trained to utilize personal protective equipment (PPE) and practice proper environmental disinfection and biosafety techniques. This includes wearing coveralls or dedicated clothing, nitrile gloves, eye protection, and a P95 or P100 respirator during bat handling and sampling. Fully Tyvek suits and HEPA-filtered Powered Air Purifying and Supplied Air Respirator Systems (PAPRs) will additionally be worn in cave systems where there is a higher risk of contact with aerosolized bat feces. All field clothing and equipment will be disinfected using Virkon disinfectant. All biological waste from field surveys will be disposed of in the appropriate container (sharps box or an autoclave bag) and will be autoclaved at local hospitals or university labs. All personnel will be vaccinated against rabies and have a neutralizing antibody titer, in accordance with WHO and CDC recommendations. Field teams will carry rabies boosters in the field and will receive a booster in the event of a potential rabies exposure.

Field safety protocol: Our procedures to deal with bites, needle-sticks etc. are as follows: The wound is washed thoroughly with soap and water to clean away dirt and debris, then vigorously scrubbed with a sterile gauze bandage and benzalkonium chloride for 5 minutes. If bleeding, pressure is applied with a sterile bandage for until bleeding has stopped. If the wound continues to bleed, medical attention at the nearest hospital is sought. The bat from which the bite or exposure originated is identified, and the samples collected from it labeled on the data sheet that these were involved in an exposure. Our procedures require that the person potentially exposed reports to a major hospital within 24 hours to

have wound examined and receive a rabies post-exposure booster vaccines as per WHO/CDC protocols. The laboratory work is lower risk, as samples placed in lysis buffer will be non-infectious. Samples placed in viral transport medium and frozen will be stored at ultra-low temperatures (-86°C) until viral isolation is required. Serum will be heat inactivated at 56°C for 30 minutes prior to testing.

Lab biosafety: The University of North Carolina at Chapel Hill, the Institute for Pathogen Biology, the Wuhan Institute of Virology, and the Wuhan University Center for Animal Experiment BSL-3 laboratories all have respective Internal Biosafety Committees and are accredited BSL-2 and BSL 3 laboratories. All experimental work using infectious material will be conducted under appropriate biosafety standards. Disposal of hazardous materials will be conducted according to the institutional biosafety regulations.

Available Treatments: No approved treatments are related for SARS and the SARSr-related bat coronavirus infections. However, therapeutic antibodies and nucleoside analogues have been successfully used in SARS-infected rodents and primates, which could be approved for compassionate use in humans exposed to the SARSr-CoV.

UNC Facilities where the select agent(s) will be used. SARS-CoV will be manipulated in research activities including establishment of viral replication curves, infection of rodent animal models and performance of plaque assays in laboratory spaces that meet operational and procedural criteria for BSL-3 activities as outlined in the CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories", 5th edition, as well as BSL-3 criteria outlined in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (April 2016). In addition, all mouse studies at UNC-Chapel Hill will be performed in an approved and registered BSL-3/ABSL-3 laboratory equipped with Techniplast Sealsafe™ HEPA-filtered animal housing for rodents. All animal protocols will be approved by the UNC-Chapel Hill IACUC.

UNC BSL-3/ABSL-3/select agent laboratories are equipped with biosafety cabinets, incubators, centrifuges with containment features, cold storage units, an autoclave, sink, eyewash and life safety equipment, and mechanical system monitors and alarms to support effective isolation and containment of operations involving SARS-CoV and SARSr-CoV. The anterooms to the BSL-3 laboratories house PAPR charging stations, laboratory and safety supplies, and a changing area. For both the BSL-2 and BSL-3 select agent spaces, access to select agents is restricted by the door between the hallway and anteroom and the door between the anteroom and BSL-3 space, requiring a combination of swipe card and punch code for entry. All select agent materials (SARS-CoV virus and genome length RNA) are stored in locked freezers and incubators.

UNC Procedures for monitoring possession, use and transfer of select agents. All personnel who will have access to select agent-regulated materials have been added to the Select Agent registration following security risk assessments prescribed by the CDC Select Agent Program. Personnel have completed training in all aspects of select agent compliance requirements and have adopted changes to standard operating procedures as applicable to assure that these requirements are met. Personnel will follow all procedures prescribed for accessing and securing the laboratory, documenting laboratory activities and materials used, and responding to incidents that could result in theft, loss, or release of select agent-regulated materials. Transfers of select agent-regulated materials will be coordinated by the laboratory managers and Responsible Official in accordance with standard operating procedures, including obtaining appropriate permits for shipping select agent materials and observing all regulations for shipping, both under dangerous goods and select agent regulations. Transfer of select agent RNA in TRIzol from registered BSL-3 to registered BSL-2 space and cDNA from registered BSL-2 space to non-registered BSL-2 space is conducted according to current select agent rules, regulations, and guidelines, including the new inactivation policies released in 2017.

UNC Biosafety, biocontainment, and security of the select agent(s). The Baric laboratories have been operational with BSL-3 core policies and procedures for ~15 years. Standard operating procedures at BSL-3 have been reviewed and approved by the UNC Chapel Hill Institutional Biosafety Committee and undergo both annual review and approval as well as updates as laboratory processes change or biosafety procedures evolve. The content of these documents has been formatted to conform to select agent regulations for the biosafety, security, and incident response plans. Additionally, lab-specific security risk assessments have been completed and recommendations implemented to ensure that security measures and procedures are sufficient to effectively minimize the possibility of unauthorized access to select agent-regulated materials. The UNC Chapel Hill facilities have undergone multiple CDC inspections and are currently in compliance with CDC requirements relating to SARS-CoV and select agent status. Our three-year renewal inspection occurred in June 2018 and we have been renewed for another three years.

UNC Biocontainment resources. All BSL-3 laboratories are under negative pressure, with redundant systems to ensure that negative pressure is maintained. All BSL-3 facilities have autoclaves to decontaminate waste materials as well as approved protocols for treatment or inactivation of any materials leaving the laboratory. All personnel are extensively trained in basic virology and safety protocols before being approved for select agent work and undergo additional extensive training to work with SARS-CoV and related SARSr-CoV as a BSL-3 pathogen. In both laboratories, annual testing is performed to verify that biosafety cabinets, laboratory supply/exhaust systems (including alarms), and other laboratory equipment are functioning as designed. The laboratories are secured at all times, and only personnel who have successfully completed Select Agent clearance and laboratory specific training requirements are permitted to enter without an escort.

P3CO Research. Recognizing the implementation of new gain of function research guidelines under P3CO, SARS-CoV and MERS-CoV are subject to these guidelines, and as such, reverse genetic studies are subject to review. Our group has considerable expertise in interfacing with the appropriate NIH P3CO institutional review boards to review, revise and finalize research designs that have the potential to modify pathogenesis or transmissibility in mammals. **Importantly, we are not proposing to genetically manipulate SARS-CoV** over the course of this proposal. However, we are proposing to genetically manipulate the full length bat SARSr-CoV WIV1 strain molecular clone during the course of the proposal, which is not a select agent, has not been shown to cause human infections, and has not been shown to be transmissible between humans.

REFERENCES

1. C. Drosten *et al.*, Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *New England Journal of Medicine* **348**, 1967-1976 (2003).
2. T. Ksiazek, D. Erdman, C. Goldsmith, A novel coronavirus associated with severe acute respiratory syndrome. *The New England Journal of Medicine* **348**, 1953-1966 (2003).
3. L. H. Taylor, S. M. Latham, M. E. Woolhouse, Risk factors for human disease emergence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **356**, 983-989 (2001).
4. Y. Guan *et al.*, Isolation and characterization of viruses related to the SARS coronavirus from animals in Southern China. *Science* **302**, 276-278 (2003).
5. W. Li *et al.*, Bats are natural reservoirs of SARS-like coronaviruses. *Science* **310**, 676-679 (2005).
6. A. Annan *et al.*, Human Betacoronavirus 2c EMC/2012-related Viruses in Bats, Ghana and Europe. *Emerging infectious diseases* **19**, 456-459 (2013).
7. S. Wacharapluesadee *et al.*, Identification of Group C Betacoronavirus from Bat guano fertilizer, Thailand. *Emerging Infectious Diseases [Internet]*, (2013).
8. S. Anthony *et al.*, Coronaviruses in bats from Mexico. *Journal of General Virology* **94**, (2013).
9. Z. A. Memish *et al.*, Middle East respiratory syndrome coronavirus in bats Saudi Arabia. *Emerg Infect Dis* **19**, (2013).
10. P. Zhou *et al.*, Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature* **556**, 255-258 (2018).
11. J. F. Drexler *et al.*, Amplification of Emerging Viruses in a Bat Colony. *Emerging Infectious Diseases* **17**, 449-456 (2011).
12. J. Huynh *et al.*, Evidence Supporting a Zoonotic Origin of Human Coronavirus Strain NL63. *Journal of Virology* **86**, 12816-12825 (2012).
13. S. K. P. Lau *et al.*, Ecoepidemiology and Complete Genome Comparison of Different Strains of Severe Acute Respiratory Syndrome-Related Rhinolophus Bat Coronavirus in China Reveal Bats as a Reservoir for Acute, Self-Limiting Infection That Allows Recombination Events. *Journal of Virology* **84**, 2808-2819 (2010).
14. P. L. Quan *et al.*, Identification of a Severe Acute Respiratory Syndrome Coronavirus-Like Virus in a Leaf-Nosed Bat in Nigeria. *Mbio* **1**, (2010).
15. S. Tong *et al.*, Detection of novel SARS-like and other coronaviruses in bats from Kenya. *Emerg Infect Dis* **15**, 482-485 (2009).
16. W. H. Li *et al.*, Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450-454 (2003).
17. X. Y. Ge *et al.*, Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).
18. V. D. Menachery *et al.*, A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nature Medicine* **21**, 1508-+ (2015).
19. N. Wang *et al.*, Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China. *Virologica Sinica*, (2018).
20. R. H. Xu *et al.*, Epidemiologic clues to SARS origin in China. *Emerging Infectious Diseases* **10**, 1030-1037 (2004).
21. L. Brierley, M. J. Vonhof, K. J. Olival, P. Daszak, K. E. Jones, Quantifying Global Drivers of Zoonotic Bat Viruses: A Process-Based Perspective. *American Naturalist* **187**, E53-E64 (2016).

22. X. L. Yang *et al.*, Isolation and Characterization of a Novel Bat Coronavirus Closely Related to the Direct Progenitor of Severe Acute Respiratory Syndrome Coronavirus. *Journal of Virology* **90**, 3253-3256 (2016).
23. L. P. Zeng *et al.*, Cross-neutralization of SARS coronavirus-specific antibodies against bat SARS-like coronaviruses. *Science China-Life Sciences* **60**, 1399-1402 (2017).
24. L.-P. Zeng *et al.*, Bat Severe Acute Respiratory Syndrome-Like Coronavirus WIV1 Encodes an Extra Accessory Protein, ORFX, Involved in Modulation of the Host Immune Response. *Journal of Virology* **90**, 6573-6582 (2016).
25. X. L. Yang *et al.*, Genetically Diverse Filoviruses in Roussettus and Eonycteris spp. Bats, China, 2009 and 2015. *Emerging Infectious Diseases* **23**, 482-486 (2017).
26. X. Y. Ge *et al.*, Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft. *Virologica Sinica* **31**, 31-40 (2016).
27. B. Hu *et al.*, Detection of diverse novel astroviruses from small mammals in China. *Journal of General Virology* **95**, 2442-2449 (2014).
28. B. Hu, X. Y. Ge, L. F. Wang, Z. L. Shi, Bat origin of human coronaviruses. *Virology journal* **12**, (2015).
29. B. Hu *et al.*, Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS pathogens* **13**, (2017).
30. C. M. Luo *et al.*, Discovery of Novel Bat Coronaviruses in South China That Use the Same Receptor as Middle East Respiratory Syndrome Coronavirus. *Journal of Virology* **92**, (2018).
31. Y. Luo *et al.*, Longitudinal Surveillance of Betacoronaviruses in Fruit Bats in Yunnan Province, China During 2009-2016. *Virologica Sinica* **33**, 87-95 (2018).
32. M. N. Wang *et al.*, Longitudinal surveillance of SARS-like coronaviruses in bats by quantitative real-time PCR. *Virologica Sinica* **31**, 78-80 (2016).
33. Z. Wu *et al.*, Comparative analysis of rodent and small mammal viromes to better understand the wildlife origin of emerging infectious diseases. *Microbiome* **6**, 178 (2018).
34. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
35. J. N. Mandl *et al.*, Reservoir Host Immune Responses to Emerging Zoonotic Viruses. *Cell* **160**, 20-35 (2015).
36. S. J. Anthony *et al.*, A strategy to estimate unknown viral diversity in mammals. *mBio* **4**, e00598-00513 (2013).
37. S. J. Anthony *et al.*, Non-random patterns in viral diversity. *Nature Communications* **6**: Article number **8147**, (2015).
38. B. Yount *et al.*, Reverse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 12995-13000 (2003).
39. Z. Lei-Ping *et al.*, Cross-neutralization of SARS coronavirus-specific antibodies against bat SARS-like coronaviruses. *SCIENCE CHINA Life Sciences* **60**, 1399 (2017).
40. V. D. Menachery, R. L. Graham, R. S. Baric, Jumping species—a mechanism for coronavirus persistence and survival. *Current opinion in virology* **23**, 1-7 (2017).
41. L. Gong *et al.*, A New Bat-HKU2-like Coronavirus in Swine, China, 2017. *Emerging Infectious Diseases* **23**, 1607-1609 (2017).
42. Y. F. Pan *et al.*, Discovery of a novel swine enteric alphacoronavirus (SeACoV) in southern China. *Veterinary Microbiology* **211**, 15-21 (2017).
43. S. K. P. Lau *et al.*, Recent transmission of a novel alphacoronavirus, bat coronavirus HKU10, from Leschenault's rousettes to pomona leaf-nosed bats: first evidence of interspecies transmission of coronavirus between bats of different suborders. *Journal of virology* **86**, 11906-11918 (2012).

44. J. H. Luo *et al.*, Bat conservation in China: should protection of subterranean habitats be a priority? *Oryx* **47**, 526-531 (2013).
45. S. Watanabe *et al.*, Bat coronaviruses and experimental infection of bats, the Philippines. *Emerg Infect Dis* **16**, 1217-1223 (2010).
46. R. L. Graham, R. S. Baric, Recombination, Reservoirs, and the Modular Spike: Mechanisms of Coronavirus Cross-Species Transmission. *Journal of Virology* **84**, 3134-3146 (2010).
47. D. Carroll *et al.*, The Global Virome Project. *Science* **359**, 872-874 (2018).
48. M. Rani *et al.*, Increased antibody affinity confers broad in vitro protection against escape mutants of severe acute respiratory syndrome coronavirus. *J Virol* **86**, 9113-9121 (2012).
49. B. Rockx *et al.*, Escape from human monoclonal antibody neutralization affects *in vitro* and *in vivo* fitness of Severe Acute Respiratory Syndrome Coronavirus. *Journal of Infectious Diseases* **201**, 946-955 (2010).
50. L. Wang *et al.*, Importance of neutralizing monoclonal antibodies targeting multiple antigenic sites on MERS-CoV Spike to avoid neutralization escape. *Journal of virology* **92**, e02002-02017 (2018).
51. J. Pallesen *et al.*, Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. *Proceedings of the National Academy of Sciences* **114**, E7348-E7357 (2017).
52. J. F. Drexler *et al.*, Genomic characterization of severe acute respiratory syndrome-related coronavirus in European bats and classification of coronaviruses based on partial RNA-dependent RNA polymerase gene sequences. *J Virol* **84**, 11336-11349 (2010).
53. K. H. Chan *et al.*, Detection of SARS coronavirus in patients with suspected SARS. *Emerging infectious diseases* **10**, 294-299 (2004).
54. P. R. Hsueh, L. M. Huang, P. J. Chen, C. L. Kao, P. C. Yang, Chronological evolution of IgM, IgA, IgG and neutralisation antibodies after infection with SARS-associated coronavirus. *Clinical Microbiology and Infection* **10**, 1062-1066 (2004).
55. V. D. Menachery *et al.*, SARS-like WIV1-CoV poised for human emergence. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 3048-3053 (2016).
56. T. P. Sheahan *et al.*, Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Science translational medicine* **9**, eaal3653 (2017).
57. A. C. Sims *et al.*, Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. *Journal of virology* **79**, 15511-15524 (2005).
58. T. Scobey *et al.*, Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 16157-16162 (2013).
59. S. Agnihothram *et al.*, Development of a Broadly Accessible Venezuelan Equine Encephalitis Virus Replicon Particle Vaccine Platform. *Journal of virology* **92**, e00027-00018 (2018).
60. S. Agnihothram *et al.*, A mouse model for Betacoronavirus subgroup 2c using a bat coronavirus strain HKU5 variant. *MBio* **5**, e00047-00014 (2014).
61. D. Deming *et al.*, Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. *PLoS medicine* **3**, e525-e525 (2006).
62. J. M. Fonville *et al.*, Antibody landscapes after influenza virus infection or vaccination. *Science (New York, N.Y.)* **346**, 996-1000 (2014).
63. K. Debbink *et al.*, Within-host evolution results in antigenically distinct GII.4 noroviruses. *J Virol* **88**, 7244-7255 (2014).
64. L. C. Lindesmith *et al.*, Broad blockade antibody responses in human volunteers after immunization with a multivalent norovirus VLP candidate vaccine: immunological analyses from a phase I clinical trial. *PLoS Med* **12**, e1001807 (2015).

65. J. A. Swanstrom *et al.*, Dengue Virus Envelope Dimer Epitope Monoclonal Antibodies Isolated from Dengue Patients Are Protective against Zika Virus. *mBio* **7**, e01123-01116 (2016).
66. X.-C. Tang *et al.*, Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution. *Proceedings of the National Academy of Sciences of the United States of America* **111**, E2018-E2026 (2014).
67. P. Daszak *et al.*, Interdisciplinary approaches to understanding disease emergence: The past, present, and future drivers of Nipah virus emergence. *Proceedings of the National Academy of Sciences*, (2012).
68. K. E. Jones *et al.*, Global trends in emerging infectious diseases. *Nature* **451**, 990-993 (2008).
69. T. Allen *et al.*, Global hotspots and correlates of emerging zoonotic diseases. *Nature Communications* **8**, 1124 (2017).

CONSORTIUM/CONTRACTUAL ARRANGEMENTS:

This project is a multi-institutional collaboration led by EcoHealth Alliance, New York (Daszak, PI), which will subcontract funds to three institutions: the Wuhan Institute of Virology (Dr. Shi), the University of North Carolina at Chapel Hill (Dr. Baric), and the Institute of Pathogen Biology (Dr. Ren). In addition, Dr. Linfa Wang from Duke-National University of Singapore (Duke NUS) will act as a senior consultant with no requested subcontract funds, and will primarily advise on the serological and molecular based diagnostic platforms. Dr. Daszak has over 15 years previous experience managing collaborative projects including two R01s on Nipah virus ecology and the current R01 on Coronavirus (AI110964) that involve multiple, separate foreign institutions; a 5-year NSF/NIH Ecology of Infectious Disease award on West Nile virus which involved multiple subcontracts, a NIAID R01 on bat viral discovery that involved multiple international contracts, and a multi-million dollar per year contract from USAID that involves 21 international partners. The applicant organization (EcoHealth Alliance) is justified in taking the lead on this project because it specializes in understanding the ecological, and virological processes underlying zoonotic disease emergence. Dr. Daszak has conducted significant preliminary work on this issue including 15-years of research on the ecological and related factors of the emergence of SARS and 15-years of work in China. The subcontract institutions will work on specific issues and areas in which they have proven expertise. These areas are:

- Human community surveillance, human clinical or hospital syndromic surveillance, CoV serology, full genome sequencing, epidemiology, and behavioral risk (Institute of Pathogen Biology, Dr. Ren)
- CoV screening and serology of non-human samples, viral pathogenesis, serological testing protocol development, host receptor binding, S protein sequencing, *in vitro* and *in vivo* virus characterization (Wuhan Institute of Virology, Dr. Shi)
- Small animal models of viral pathogenesis, primary human cell cultures, CoV reverse genetics, reconstruction of zoonotic CoV (University of North Carolina at Chapel Hill, Dr. Baric).

Dr. Daszak has had inter-institutional contractual agreements with the Wuhan Institute of Virology for over 13 years. Drs. Shi and Daszak have collaborated together since 2002 and have been involved in running joint conferences, collaborating on papers, and shipping samples into and out of China. Drs. Baric, Shi, Wang, and Daszak have collaborated closely for over 10 years on Coronavirus and other emerging disease research. Drs. Shi and Ren have collaborated on viral discovery projects 8 years.



WUHAN INSTITUTE OF VIROLOGY THE CHINESE ACADEMY OF SCIENCES

Address: Xiaohongshan 44, Wuchang, Wuhan 430071, Hubei, P.R. China
Tel: +86-27-87199162 Fax: +86-27-87199162 <http://www.whiov.ac.cn>

31 October 2018

Dr. Peter Daszak
President
EcoHealth Alliance
460 W 34th St. 17th Floor
New York, NY 10001 USA

Dear Dr. Daszak,

The Wuhan Institute of Virology, Chinese Academy of Sciences, has an high interest in working with EcoHealth Alliance and its scientists to identify and prevent the transmission of bat coronaviruses to human populations globally. In particular, the NIAID funded R01 renewal proposal entitled "Understanding the risk of bat coronavirus emergence" will provide an excellent opportunity to achieve these goals.

The Wuhan Institute of Virology, Chinese Academy of Sciences, recognizes the mutual benefits to be gained through research cooperation and a successful partnership with EcoHealth Alliance in the field of identification and prevention of zoonotic disease transmission. It is vital to not only identify the diseases themselves, but also identify high-risk human populations and the actions that put them at risk for infection along with evaluating approaches to intervention and disease management.

Understanding and preventing exposure and transmission of zoonotic diseases from wildlife to humans remains a high priority for prevention of pandemics. In our discussion with EcoHealth Alliance, we have agreed to participate in activities that will strengthen the ability of China and other countries in the region to respond to epidemic disease outbreaks - particularly those of animal origin. To assist in this study, we will provide participating laboratories in China with human samples both new and archived and support research in bat coronaviruses.

We at Wuhan Institute of Virology, Chinese Academy of Sciences, look forward to our continued collaborations with the EcoHealth Alliance team and working further on this worthwhile study.

Sincerely,


Dr. Yan Yi Wang
Director, Wuhan Institute of Virology
Chinese Academy of Sciences
Xiao Hong Shan, No.44
Wuhan 430071 China
(b) (6)



中国医学科学院 病原生物学研究所 北京协和医学院

01 November 2018

Dr. Peter Daszak
President
EcoHealth Alliance
460 W 34th St. 17th Floor
New York, NY 10001 USA

Dear Dr. Daszak,

The Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College (IPB, CAMS&PUMC) has an high interest in working with EcoHealth Alliance and its scientists to identify and prevent the transmission of bat coronaviruses to human populations globally. In particular, the NIAID funded R01 proposal entitled "Understanding the risk of bat coronavirus emergence" will provide an excellent opportunity to achieve these goals.

The IPB, CAMS&PUMC recognizes the mutual benefits to be gained through research cooperation and a successful partnership with EcoHealth Alliance and long term colleague Dr. Zhang Shu-Yi in the field of identification and prevention of zoonotic disease transmission. It is vital to not only identify the diseases themselves, but also identify high-risk human populations and the actions that put them at risk for infection along with evaluating approaches to intervention and disease management.

Understanding and preventing exposure and transmission of zoonotic diseases from wildlife to humans remains a high priority for prevention of pandemics. In our discussion with EcoHealth Alliance, we have agreed to participate in activities that will strengthen the ability of China and other countries in the region to respond to epidemic disease outbreaks – particularly those of animal origin. To assist in this study, we will provide participating laboratories in China with human samples both new and archived and support research in bat coronaviruses.

We at IPB, CAMS&PUMC look forward to our continued collaborations with the EcoHealth Alliance team and working further on this worthwhile study.

Sincerely,

Lili Ren
Co-Investigator
Institute of Pathogen Biology,
Chinese Academy of Medical Sciences & Peking Union Medical College
No.9 Dong Dan San Tiao, Dong Cheng District
Beijing, 100730 P.R.China
E-mail: (b) (6)





UNC
GILLINGS SCHOOL OF
GLOBAL PUBLIC HEALTH

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

DEPARTMENT OF EPIDEMIOLOGY 1 919-966-2089
MCCAVRAN-GREENBERG HALL
CAMPUS BOX 7435
CHAPEL HILL, NC 27599-7435

October 24, 2018

Peter Daszak, PhD
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

Reference: *Program Announcement number PA-18-484, entitled NIH Research Project Grant (Parent R01 Clinical Trial Not Allowed), dated December 6, 2017*

Dear Dr. Daszak,

This letter confirms that the appropriate program and administrative personnel at The University of North Carolina at Chapel Hill (UNC-CH) have reviewed the above referenced program announcement and are committed to enter into a subcontract with the EcoHealth Alliance for the performance period of July 1, 2019 to June 30, 2024. The work to be performed by UNC-CH does not include animal and/or human research subjects. UNC-CH maintains an active and enforced conflict of interest policy meeting the requirements of 42 CFR Part 50, Subpart F and 45CFR Part 94.

EcoHealth Alliance's Principal Investigator on this proposal is Dr. Peter Daszak. The UNC-CH budget, budget justification and scope of work are provided as separate enclosures to this letter. The estimated cost of the proposed subcontract will not exceed \$388,750 and includes appropriate direct and indirect costs.

Furthermore, by submission of this commitment letter UNC-CH and its Principal Investigator (PI) certify (1) that the information submitted within the application is true, complete and accurate to the best of the UNC-CH's and PI's knowledge; (2) that any false, fictitious, or fraudulent statements or claims may subject the UNC-CH and PI to criminal, civil, or administrative penalties; and (3) that the PI agrees to accept responsibility for the scientific conduct of the project and to provide the required progress reports if an award is made as a result of UNC-CH's application.

If you have any questions, please contact the undersigned at 919-966-3895.

Sincerely,

Terry Magnuson, PhD
Vice Chancellor for Research

Ralph S Baric, PhD
Professor, Epidemiology, Microbiology and Immunology

Enclosed:
Budget
Budget Justification
Scope of Work



31 Oct, 2018

Dr. Peter Daszak
EcoHealth Alliance
460 West 34th Street, Suite 1701
New York, NY 10001, **USA**

Dear Peter,

I am writing this letter in strong support of the proposed renewal of the NIH (R01AI110964, Understanding the Risk of Bat Coronavirus Emergence) project led by EcoHealth Alliance.

As you know, I have long experience with EIDs associated with bats, having worked with Hendra virus in Australia, Nipah virus in Malaysia, Singapore and Bangladesh, SARS related viruses in China, Reston ebolavirus in the Philippines. More recently, in collaboration with scientists in EcoHealth and China, I played an important leadership role in coordinating and directing the research which discovered a bat HUK2-related coronavirus as the causative agent of a major swine acute diarrhea syndrome (SADS) outbreak in Southern China.

Your current proposal complements and substantially expands this approach in promising novel and more powerful tools to understand how host immune dynamics and heterogeneity in immune response affect the timing, location, and severity of disease outbreaks in wildlife, and risk of spillover from wildlife to human populations.

I very much looking forward to participating in this initiative and should EcoHealth Alliance be successful in its application for renewal, I agree to participate in activities associated with this project, including contributing expertise, helping identify, locate and interpret relevant data, and participating in partner meetings.

This letter conveys my strong interest and commitment to making this initiative a success. I am excited to be part of the initiative to develop tools to better interrogate seroprevalence data and better predict zoonotic disease emergence. I'm confident that the approach will improve our understanding of the dynamics of infectious diseases and have wide application in public health, and I look forward to working with EcoHealth Alliance on this project

Yours sincerely,

A handwritten signature in black ink, appearing to read "Linfa Wang".

Linfa (Lin-Fa) WANG, PhD FTSE
Professor & Director, Programme in Emerging Infectious Diseases

8 College Road, Singapore 169857
T 6516 7666 F 6221 7396
www.duke-nus.edu.sg

A school of the National University of Singapore (RCB No: 200604346E)

RESOURCE SHARING PLAN

Data Sharing Plan: Data will be available to the public and researchers without cost or registration, and be released under a CC0 license, as soon as related publications are in press. Data will be deposited for in long-term public scientific repositories – all sequence data will be made publicly available via GenBank, species location data via the Knowledge Network for Biodiversity, and other data will be deposited in appropriate topic-specific or general repositories. Computer code for modeling and statistical analysis will be made available on a code-hosting site (GitHub), and archived in the Zenodo repository under an open-source, unrestrictive MIT license. Limited human survey and clinical data will be released following anonymization and aggregation per IRB requirements. Publications will be released as open-access via deposition to PubMed commons.

Viral isolates will remain at the Wuhan Institute of Virology initially. Isolates, reagents and any other products, should they be developed, will be made available to other NIH-funded researchers via applicable Wuhan Institute of Virology and EcoHealth Alliance Material Transfer Agreements and/or licensing agreements.

Sharing Model Organisms: We do not anticipate the development of any model organisms from this study. Should any be developed, they will be made available to other NIH-funded researchers via applicable Wuhan Institute of Virology and EcoHealth Alliance Material Transfer Agreements and/or licensing agreements.

Genomic Data Sharing: We anticipate obtaining genetic sequence data for 100s of novel coronavirus genotypes, including RNA-dependent RNA polymerase (RdRp) and Spike genes for all strains/genotypes. In addition, we will generate full viral genomes for a subset of the bat SARSr-CoVs that we identify. All sequence data will be deposited in the NIH genetic sequence database, GenBank. We will ensure that all meta-data associated with these sequences, including collection locality lat/long, species-level host identification, date of collection, and sequencing protocols will also submitted. The genotype data will be made publicly available no later than the date of initial publication or six months after the receipt of final sequencing data, whichever comes first. We anticipate sequence generation will occur over the 5 year proposed project period.

Genome Wide Association Studies (GWAS): Not applicable.

Rigor and Authentication of Key Biological and/or Chemical Resources

Our project aims to understand the risk of bat SARS related coronavirus (SARSr-CoV) disease emergence in people, and will use some non-standard biological and chemical resources that require validation and authentication. We will construct chimeric SARSr-CoVs using a WIV1 backbone and the S genes of selected SARSr-CoV strains, and assess capacity to infect hACE2, bACE2 and cACE2 Vero cells, HeLa cells, primary human airway epithelial cells, and potentially CaCo cells for HKU3r-CoVs (which have not yet been cultured in human cell lines and may use intestinal epithelium in nature). We will then conduct experimental infections in hACE2 transgenic mice to assess pathogenicity and clinical signs. Each of these methods have been previously validated and published by our collaborative research team, as highlighted in the research proposal. Each laboratory-based research partner in our project (including University of North Carolina (UNC), Wuhan Institute of Virology (WIV), Institute of Pathogen Biology (IPB) at Chinese Academy of Medical Sciences, and Duke National University of Singapore (DukeNUS)) each have specialized strategies to oversee the authentication of key biological resources, reagents and chemical resources. EcoHealth Alliance will actively engage with each partner to ensure that the highest quality science, public accountability, and social responsibility in the conduct of science are maintained throughout. The overall goal is to ensure that the underlying scientific foundation of the project from conception to completion is scientifically sound. To ensure scientific rigor (e.g., determining group sizes, analyzing anticipated results, reducing bias, ensuring independent and blinded measurements, improving precision and reducing variability including or excluding research subjects, and managing missing data), EcoHealth Alliance will review scientific approaches and outcomes throughout the duration of the award. We will ensure that experimental designs will include considerations of sex as a “Relevant Biological Variables” in all studies involving human subjects or vertebrate animals. Unless otherwise specified and justified, all experiments will include male and females. UNC and WIV will implemented an “audit trail” that tracks animals used in experimental investigations (Aim 3) from parents, through birth, shipment, experimentation, results, QC and analyses, providing outside researchers the ability to track experiments from conception through publication.

Cells.

Early passage primary human airway epithelial cells are a key reagent for the proposed studies. Human lung cells are derived from donors of both sexes and from all ages and ethnic groups. Care is taken during cell isolation to only handle one human organ at a time. Similarly, primary cell populations are handled carefully, only one donor cell type from a single donor at a time to avoid any mixing. The cells are observed to exhibit well-described prototypical characteristics of human primary lung cells in cell type specific medias in culture. For quality control, the cells are cultured in antibiotic free media to test for bacterial and fungal microbial contamination and are subjected to mycoplasma testing. Once the epithelial cells are grown as polarized and differentiated monolayers, a representative sample is subject to quality control histological analysis of cell morphology and Short Terminal Repeat (STR) marker profiling by the UNC Lineberger Cancer Center’s Tissue Culture Facility (TCF). Routine evaluations for mycoplasma contamination are routinely performed in the laboratory.

- Certain experiments also employ immortal cell lines. Cell lines are obtained from the ATCC, or from the TCF. The TCF maintains cell lines, utilizing STR marker profiling and records of authentication are available. New cell lines not available directly from the TCF can be authenticated through the STR marker service provided by the TCF. Cell lines are routinely evaluated for mycoplasma contamination.
- When receiving cell lines, lab members initially maintain isolation and keep them isolated from other authenticated cell lines until mycoplasma testing and STR marker profiling is performed. All cell lines must be authenticated before commencing experimental work with them.
- Records are maintained for each of the cell lines regarding 1) the origin of the cell lines; 2) when they were resuscitated; 3) number of passages; 4) all test results; 5) any unique distinguishing growth behavior; and 6) any known genetic features.
- Cells that have been passaged for 6 months after receipt or from resuscitation will be re-authenticated, or a new vial of the working stock will be thawed.
- Lab members routinely examine cultured cell morphology by phase microscopy and monitor the growth characteristics in culture. New vials of the working stock are thawed if deviation from the baseline is

observed.

- Mycoplasma contamination is re-checked whenever cells are extensively passaged to create new stocks.

Animals (Mice)

- **Rodent genotyping for mouse strain genetic validation.** Inbred mouse strains are an invaluable tool for biomedical research, and hACE2 transgenic mice represent a key aspect of Aim 3 to assess pathogenicity and clinical signs for SARS-CoVs. To ensure that the genetic background of all mice used within this program is known and when applicable they are part of a known inbred strains, we will genotype each mouse strain used within this program on the appropriate MUGA platform (Morgan, AP et.al., G3 2016, Dec 18). The most recent iteration of this state of the art genotyping array contains over 140,000 markers and can be used to precisely determine the genetic background at the substrain level and the precise location (at <1 megabase resolution) of genomic regions derived from different mouse inbred strains. In this way, the identity and genomic integrity of all mice used within these studies will be ensured. As new diagnostic assays become available, we will assess their utility and cost effectiveness the different MUGA arrays and implement them as appropriate.
- Furthermore, for each mutant mouse strain used within the project, positive diagnoses of the mutation will be assessed for each cohort of experimental animals with a diagnostic validated PCR assay or Sanger sequencing diagnostic to ensure proper results.

Recombinant and Wildtype Viruses and Mutant Derivatives.

- Recombinant and wildtype viruses contain unique marker mutations that allow for distinguishing strains and mutation profiles, using a combination of full genome sequencing, reverse transcription-polymerase chain reaction (RT-PCR) or RT-PCR restriction fragment length polymorphism analyses (RT-PCR RFLP). Our group has developed defined primer pairs to distinguish between SARS-CoV and SARS-related bat coronaviruses as well as MERS-CoV and MERS-related bat coronaviruses. All viruses will be validated and certified pure of contaminating viruses prior to use or shipment to other laboratories.