Protein Expression and Purification

For protein expression and purification, we request a total of $6,590 in year 1, $6,705 in year 2, and $5,260 in each year of years 3-5. Details of protein expression and purification costs include histidine tagged protein purification columns ($116 each), Q sepharose fast flow media ($116 each), protein inhibitor ($289 each), eStain Protein stain and SP sepharose fast flow ($116 each), and bacterial cell tissue culture plates ($116 each). It is expected that most samples will be processed in year 1, so costs are estimated to reduce by 20% in years 2-5.

Serological Tests

We request support for serology assays. None will be conducted in year one until we have samples. Costs are estimated for years 2-5 with $9,220 in year 2 and due to additional IgM and IgG secondary antibodies costs and increased sample testing, $13,436 in years 3-5. Cost estimates include IgM and IgG secondary antibodies $72.25; mouse and rabbit IgG antibodies at $434; mouse and rabbit IgM antibodies at $434; and ELISA plates at $2.90 each.

Lab Supplies

Funding is requested to support laboratory supplies including three (3) -80°C freezers ($1,816 each); reagents including agarose, sodium chloride, yeast extract, phosphate buffer, Tris and other biochemical reagents (average costs for reagents and large tube supplies are estimated to increase in years 2-5 (average cost $665 per year); disposable personnel protective equipment (PPE), portal breathing apparati (PAPR), gloves and protective clothing to be used in BSL settings are estimated at $723 per years 1 and 2 ($867 each); and *in vitro* culture (lipofectamine2000) will be required at cost of $434 in year 1 and $2,168 per year in years 2-5.

H. Indirect Costs

We are requesting an extremely low indirect cost of 8% on all direct costs.

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RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Personnel	
Section B, Other Personnel	
Total Number Other Personnel	0
Total Salary, Wages and Fringe Benefits (A + B)	85,000.00
Section C, Equipment	0.00
Section D, Travel	
1. Domestic	0.00
2. Foreign	21,570.00
Section E, Participant/Trainee Support Costs	0.00
1. Tuition/Fees/Health Insurance	0.00
2. Stipend	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other	0.00
6. Number of Participants/Trainees	
Section F, Other Direct Costs	243,430.00
1. Materials and Supplies	
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other 1	0.00
9. Other 2	0.00
10. Other 3	0.00
Section G, Direct Costs (A thru F)	350,000.00
Section H, Indirect Costs	28,000.00
Section I, Total Direct and Indirect Costs (G + H)	
Section J, Fee	0.00
Section K, Total Costs and Fee (I + J)	378,000.00

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1. Vertebrate Animals Section

Are vertebrate animals euthanized?

If "Yes" to euthanasia

Is the r...

○ Yes   ○ No

If "No" to AVMA guidelines, describe method and pro... scientific justif...

2. *Program Income Section

*Is ...

○ Yes   ● No

If you checked "yes" abov... cating that program in...
source(s)...

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3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells?   ○ Yes   ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❏   Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

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4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes   ● No

If the answer is "Yes" then please answer the following:

*Previously Reported:

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5. Change of Investigator/Change of Institution Section

❏   Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❏   Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

Introduction

1. Introduction to Application	
(for Resubmission and Revision applications)	

Research Plan Section

2. Specific Aims	NIAID_COV_2019_SPECIFIC_AIMS_final.pdf
3. Research Strategy	NIAID_COV_2019_RESEARCH_STRATEGY_final.pdf
4. Progress Report	

Other Research Plan Section

5. Vertebrate Animals	NIAID_COV_2019_VERTEBRATE_ANIMALS_Final
6. Select Agent Research	NIAID_COV_2019_SELECT_A
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	NIAID_COV_2019_LOS_Final.pdf
10. Resource Sharing Plan(s)	NIAID_COV_2019_RESOURCE_SHARING_PLAN_Final.pdf
11. Authentication of Key Biological and/or Chemical Resources	

Appendix

12. Appendix	

SPECIFIC AIMS

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in 2002. [...] the continued spread of Middle East Respiratory Syndrome [...] (MERS) [...] our group as bat species, and since then we have sequenced dozens of novel SARS-related CoV (SARSr-CoV) strains. Our previous R01 work demonstrates that bats in southern China harbor an extraordinary diversity of SARS [...] of which are able [...] use human ACE2 to enter into human [...] can infect humanized mouse models to cause SARS-like illness, and evade available therapies or vaccines. We found that [...] markets, and that people living close to bat habitats are the primary [...] groups for spillover. At one of these sites [...] we found diverse [...] SARS [...] of human exposure among people living [...] SARSr-CoVs, and of public health impacts. Yet [...] and sites that **harbor CoV strains with high** [...] human spillover? Which human behaviors [...] risk of bat SARSr-CoV exposure that [...] lead to infection? Does human exposure to [...] like or other illness? Can we characterize viral strain diversity, bat traits and human behav [...] potential future CoV spillover? The proposed work in this renewal R01 build on these findings to address these iss [...] by conducting: 1) focused sampling of bats in south [...] to identify strains [...] **predicted risk of spillover; 2)** community-based, and clinic-based syndromic sampling of people to identify **spillover, and assess** [...] **viral risk factors and evidence** [...] conduct [...] viral characterization and analyze epidemiological data **to identify hotspots of future CoV spillover risk**. This work will follow 3 specific aims:

Aim 1: Characterize the diversity and distribution of [...] spillover [...]
Chi [...] We will conduct targeted [...] sampling at sites where [...] predict [...] undiscovered high risk SARSr-CoV strains exist. Bat sampling [...] will be targeted geographically and by host species to test pre [...] evolutionary diversity of S [...] analyze [...] spillover to people in Aim 3.

Aim 2: Community- and clinic-based [...] SARS [...] spillover [...] **and potential public health consequences**. We will conduct focused, targeted human surveys and sampling to identify key risk factors for SARSr-CoV spillover and evidence of illness. To maximize our opportun [...] capturing human exposure to bat CoVs, we will c [...] [...] with high SARSr-CoV [...] and diversity [...] seropositive stat [...] [...] about human whic [...] conduct clinic-based syndromic surveillance close to [...] sites to identify patients p [...] like illness and severe acute respiratory illness, assess th [...] [...] samples for PCR and serological evidence of SARSr-CoV infection. We will conduct follow-up sampling to capture patients who had not yet se [...] ed at the [...]

Aim 3: In vitro and in vivo characterization of SARSr-CoV [...] with phyloge [...] **phylogenetic** analyses to identify the regions and viruses of public health concern. We [...] the propensity of novel SARSr-CoVs to infect people in vitro using primary human airway epithelial cells, and in vivo using the transgenic hACE2 mouse model [...] that SARSrCoV [...] with 10-25% divergence in S protein [...] human cells, and to evade mAb therapeutics and vaccines. We will then map the geographic distribution of their bat hosts and other ecolog [...] factors to identify the key 'hotspots' of risk for future spillover [...]

Overall, our SARSr-CoV program [...] factors contributing to CoV emergence while informing high impact strategies to intervene and prevent future pandemics. This includes providing c [...] reagents, ther [...] future SARSr-CoV pandemic a [...] [...] health prepared [...]

previous work also suggests that SARSr-CoVs with S proteins that are ~10% divergent from SARS-CoV resist neutralization by therapeutic mAbs and escape SARS-CoV vaccines (*17, 18, 23*), suggesting that . However, viruses with 10-25% divergence in S proteins may bind to human cell receptors, but completely evade therapeutic and vaccine effects, and could therefore be a higher risk for public health. We will sequence the S proteins of novel SARSr-C for experimental work in Aim 3 to test

1.2 General A sequence data from our previous to pinpoint sites and ho Guizhou and Guangdong undiscovered SARSr-CoVs and with competent hosts. Precise sampling site locations will be refined Y1. We will target at least 5,000 individual bats over 5 years from 15 *Rhinolophus* bats, which calculate will allow us to almost fully character ected natural diver SARSr- and other p-CoVs in the region. B will be captured, sampled, and released at the site. Specimens will be transported in liquid N to Wuhan Inst.Virology (WIV) for PCR screening, and positive samples selected for further molecular charac zation and S Protein sequencing. EHA will lead the study d and data analysis for this Aim; and WIV will lead the testing and viral sequencing.

1.3 Sampling and testing of bats: 1.3.a Site selection & viral trait modeling, phylogeographic analyses of RdRp and S Protein sequences, and geographic and host species-based viral discovery curve analyses to identify SARSr-CoV diversity hotspot reg We will sample at 8 new sites in 4 provinces. We will use cave site data and demographic information to identify two sites in each of Yunnan, Guangxi, Guangdong, humans likely have contact with bats. In Yunnan, we will identify two sampled caves close to, but distinct from, the This will provide adequate coverage of lineage 1 and 2 SABS-CoVs, including a rich source of new HKU9-CoVs, which have unknown potential for zoonotic spillover. Sampling will begin WIV will use survey data fro our previous PCR, host species, popula 10 under-sampled *Rhinolophus* spp., 5 SARSr-CoV positive in our study, and a small number of related bat genera (including *Hipposideros* spp. and *Aselliscus* spp.) we previously found PCR + for SARSr-CoVs **(Table 1)**. We will sample at least 5 bats from these 4 province ~5-12% prevalence of SARSr-CoV in *Rhin* **us 425 (±175) positive individual bats, and ~125 novel strains.**

1.3.b CoV screening isolation: Viral RNA A will be extracted pellets/each swabs with High Pure Viral RNA Kit (Ro A will be aliquoted and stored at 80C. One-step will be used to detect the presence of CoV sequences using primers that target a 440-nt fragment in the dependent RNA polymerase gene (RdRp) of all known α- and β-CoVs (45). PCR products will and sequenc with an ABI DNA sequencer. We will attempt isolation on samples with diverse and interesting novel CoVs, using Vero E cells and bat primary cell culture

1.3.c Sequencing S prot: For all novel SARSr-CoV strains, we will sequence the complete S gene by amplifying overlapping fragments using degenerate primers as shown previously 29 Full-length genomes of selected SARSr-CoV strains (representative across subclades) will be sequenced via high throughput sequencing method followed by genome walking. The sequencing libraries will be constructed using NEBNext Ultra II DNA Library Prep PCR will be performed to fill gaps in the genome. The full length S gene sequences, including the amount of variation in the S receptor binding residues that bind the ACE2 receptor, will be used to select strains for Aim 3 experiments.

1.3.d Host ACE2 receptors: We will sequence host ACE2 receptors of different bat species or different populations from a single species (e.g. *Rhinolophu* lative importance high risk SARSr-CoVs a across bat and human orthologue ACE2 contact interface residues that engage the SARS RBD as a potential indicator of SARSr-CoV cross species transmission potential

1.4 Analyses: 1.4.a Bat-CoV evolution an phyloge analyses of RdRp, Spike, and full genome sequence (when availa e) data to reconstruct the evolutionary history of the novel bat SARSr ident

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reconstruct β-CoV evo[...] using our expanded data[...]. Ecological niche models will be used to predict spatial distribution [...] positive species, [...] overlap with people. We will use our viral discovery/accumulation curve approach **(Fig. 4)** to monitor progress toward discovery of [...] of predicted diversity of SARSr-CoVs (lineage [...]) within specific [...] halting [...] when this target is reached, and freeing [...] resources for work [...]

1.4 h Viral strain prioritization [...] projected 100-200 novel SARSr-CoV strains, we will down-select [...] prioritize for further characterization b[...] on [...] are: i) different from SHC014, WIV1, SARS-CoV with diversity ranges of 10-25%; ii) have virus S RBD [...] human/bat receptors iii) have [...] recombinant chimeric spikes indicative of [...] between clade 1 and II strains; iv) have bat ACE2 receptors that might select for spike RBDs that can use [...] an receptors for entry (15/18 conserved residues in human/bat ACE2 molecules that bin [...] SARSs-CoV S RBD domains are likely more e[...] ent receptors than 3/18 conserved sites). Using structural models based on the SARS S gl[...] the [...] location of antigenic variation will be a[...]ted onto the structure, furth[...] [...]cations of highly evolving and conse[...]

1.5 Potential problems/alterna[...]

select [...] We have a [...] [...] the start of this project. Due to the abundance of distinct caves in the region [...] we will rapidly identify a suitable, complementar[...] [...] with similar bat speci[...] composition and [...] **may [...]fy β-CoV[...] in our sample ba[...]** [...] due to seasonalit[...] viral shedding. The sam[...] regions we have [...]ected are subtropical and [...] [...] data, and even published studies in temperate regions (52), do not suggest a strong pattern of seasonality in SARSr-CoV shedding. Nonetheless [...] for this we will conduct sampling ev[...]y on quarterly basis within each province.

Aim 2: Community and clinic-based surveillance to capture SARSr-CoV spillover, routes of exposure and potential public health consequen[...]

2.1 Rationale: Our previous R01 study identified serological evidence of exposure to SARSr-CoVs in certain communities in S. China **(Table 2, Fig. 10)** (19). Howe[...] e low seroprevalence (0.6% [...] sites) suggests we need a larger sample size, and a focused, targeted study to maximize the likelihood of identifying seropositive cases. In Aim 2, we will use combined biological-behavioral risk surveillance in targeted populations within the community and clinical settings to 1) identify risk factors co[...] [...]vity (exposure to) and PCR positive status (infection with) bat SARSr-CoVs, and 2) assess possible health effects of SARSr-CoVs in[...] likelihood of recent 'hidden' spillover events and their link to health [...] as well as the risk [...] emergence of SARS-like diseases. It will also support the development of risk mitigation strategies by public health authorities [...]

2.2 General Approach/Innovat[...] We will use a dual study design to gain in-depth understanding of exposure and risk fac[...] SARSr-CoV spillover **(Fig. 13)**. We will conduct community-based surveillance with more focused questionnaires and biological sampling to determine the seroprevalence of SARSr-CoVs in at-risk human populations, and to identify risk factors for SARS-CoV spillover in these communities. We will conduct clinic-based syndromic su[...] and biological samp[...] sites that include the [...]unity-based surveillance sites within their catchment. This will include follow-up sa[...] [...] measure serc[...]onversi[...] [...]lly infected people and the follow-up of contacts. We will [...]PCR to test for current infection [...] SARSr-CoV replication. Both community-based and clinic-based syndromic surveillance programs are case-control studies designed with the sample sizes ne[...]ssary to statisti[...]lly [...] with [...] power of 80% risk factors and health impacts for SARSr-Co[...] spillover, linked to s[...]

2.3 Targe[...] population & sample size: We will target sites in the same four provinces, and close to those for [...] the S protein sequence divergence of 5-25% and 6) high[...] rates of human-wildlife interactions. Community-based surveillance will be conducted at 2 sites in each of the 4 provinces, a total of 8 sites. From our previous work we anticipate that 10-30% of the community population will have [...] exposure [...]

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therefore likely to have infected people more widely. Incoporating serological testing for SHC014 is also likely to yield higher seroprevalence because it is readily divergent from SARS-CoV wildtype and therefore unlikely to have been picked up in our earlier testing. It is possible that non-neutralizing but protective epitopes exist that afford an accurate measure of cross reactivity between clade1 and clade 2 strains. This information would allow us to target exposure to strains with 15-25% divergence from SARS-CoV. Additionally, we will test samples for antibodies to common human CoVs (HCoV NL63, OC43 – see page __). To serologically target novel reservoir exposure, we will conduct: 1) ELISA screening with SARSr-CoV S or RBD; 2) confirm these results by Western blot; then 3) use NP based ELISA and LIPS assays to assess the diversity of SARSr-CoVs. For the NP ELISA, micro-titer plates will be coated with 100 ng/well of recombinant batCoV NP and incubated with human sera in duplicates followed by detection with HRP labeled goat anti-human IgG antibody. positive samples will be subjected to Western blot at a dilution of ... using ... we will use an S protein-based ELISA to distinguish the lineage of SARSr-CoV. As new SARSr-CoVs are discovered, we will rapidly design specific Luciferase Immunoprecipitation system (LIPS) assays targeting the S1 genes of bat-CoV strains for follow-up serological surveillance, as per our previous work (18, 57).

2.6.b RT-PCR testing. Specimens will be screened using RT-PCR for the Henipavirus (see section 2.5.2 for details). Positive samples will be subjected to full genome sequencing and RT-PCR amplification of the S glycoprotein gene. Samples from the clinic-based surveillance will be validated with PCR primers specific for Influenza A & B, HCoV NL63, OC43, HKU1, SARS-CoV & 229E sample sets, and SARS-CoV and HKU10 as an opportunistic survey for potential spillover these CoVs as proposed ...

2.7 Epidemiology. We will conduct a case-control study to identify risk factors for SARSr-CoV spillover. "Cases" are defined as participants whose samples tested positive for SARSr-CoVs by serological ... "Controls" will be selected from the pool of participants that will be enrolled during sampling ... will use nearest neighbor matching to pair cases demographically ... will use generalized linear models to analyze correlation between serological/PCR status and risk factors including: Activities with likely exposure to 1) bats, 2) livestock, and 3) locations of residence and work. We will ... unexposed enrollees, in the time course of illness, severity of symptoms, and type of symptoms.

2.8 Potential problems and alternative approaches. Potential problem: we may not be able to identify sufficient seropositives to statistically analyze risk behavior or illness. First, we are focusing our community-based surveillance to subpopulations with high-levels of bat exposure, at sites selected for diverse and prevalent SARSr-CoVs, and are adding clinic-based syndromic surveillance of SARI and ILI cases in these same regions – both will increase likelihood of finding positive individuals. Secondly, our assays will include a panel of assays for a large diversity of lineage 1 and 2 SARSr-CoVs as well as SADS-CoV, HKU10 and other bat-borne CoVs. *Rhinolophus* spp. bats host all of these (overall bat CoV PCR prevalence, 11.0%, β-CoV, 3.4% ... CoV, ... these specimens ... shed light on behaviors that predispose to CoV spillover ... in our panel, so that even if low prevalence of bat CoVs ... sectional study of the seroprevalence of normal human ... ... will also allow us to analyze human-wildlife contact for our cross-survey work. We will analyze intensity of contact against other risk factors and clinical outcomes to provide useful proxy information for spillover risk. Patients visiting clinics may have **cleared virus, but not yet developed IgG antibody responses** ... sampling should ... This is because the maximum ... 28 days (53). We also expect that patients in rural communities will only visit clinics when symptoms have progressed, likely coinciding with late illness and onset of IgG. We will also have data from our community study, so won't be completely dependent on clinic-based serology ... outweighed by the potential public health importance ...

Serological ...
tiered serological testing system outline in 2.6.a to identify these novel CoVs, however, we will also remain flexible on interpretation of data to ensure a broad approach to recombinant ...

Aim 3: *In vitro* and *in vivo* characterization of SARSr-CoV spillover risk, coupled with spatial and phylogenetic analyses to identify the regions and viruses of public health concern

3.1 Rationale/Justification: In Aim 1, we aim to expand the known diversity of SARSr-CoV strains, targeting 10-25% S protein divergence that we predict infers high spillover risk and evasion of immune-therapeutic and vaccine efficacy. In **Aim 3**, we will further characterize the zoonotic potential of a selected group of these novel SARSr-CoVs, using infectious clone technology, in vitro and in vivo infection experiments and analysis of HKU3r-CoV receptor binding to test our predictions of which spike protein sequence thresholds predict spillover potential (*18, 55*). We will analyze data gathered in these viral characterization and infection experiments, coupled with bat-host distribution, viral diversity and phylogeny, as well as human survey behaviors and illnesses, and human serology to assess spillover risk of these SARSr-CoVs in different bat species across southern China. This will enable us to prioritize regions for public health intervention and surveillance to prevent the emergence of a novel SARSr-CoV.

3.2 General Approach: We will use S protein sequence data to identify bat SARSr-CoVs with 10-25% S protein divergence we predict as high public health potential and construct chimeric SARSr-CoVs using the WIV1 backbone and these S genes with a goal of testing receptor binding and host range, and assess infection of non-permissive cells expressing human, bat and civet ACE2 receptors, Vero cells, primary human airway epithelial cells, and CaCo cells for HKU3r-CoVs, which have not been cultured and may use intestinal epithelium in nature). We will conduct experimental infections in hACE2 transgenic mice to assess pathogenicity and clinical signs (*18*). Finally, using a panel of mAbs that neutralize SARS-CoV, and that *in vitro* and *in vivo* and vaccine strains of SARS-CoV, we will attempt to identify potentially divergent S protein sequences to evade therapeutics, revealing strains with high public health potential. We will also conduct limited experiments to analyze HKU3r-CoV receptor binding to assess spillover potential. Using these results, and data from Aims 1 and 2, we will use spatial modeling techniques to identify geographic hotspots in southern China where bat species that harbor high risk SARSr-CoVs inhabit, where communities that have high exposure to bats exist, where serological or PCR evidence of spillover has been identified, and where underlying demographic or environmental trends suggest high risk of future emergence.

3.3 Virus characterization: 3.3.a Construction of chimeric SARSr-CoV viruses: Infectious clones along with the S gene of novel SARSr-CoVs and the SARSr-CoV WIV1 genome backbone using the reverse genetic system developed in our previous R01 (*24*). The correct infectious BAC clones will be screened by BAC DNA digestion with appropriate restriction enzymes and PCR amplification. The S gene in each recombinant will be amplified and then verified by sequence analysis. Our research group is well versed in coronavirus reverse genetics.

3.3.b Cell entry analysis: HeLa cells expressing human ACE2 are cultured on coverslips in 24-well plates and incubated with the chimeric bat SARSr-CoVs with different spike proteins at a multiplicity of infection (MOI) = 1.0 for 1h. The inoculum is removed and the cells are washed twice with PBS and supplemented with medium. HeLa cells without ACE2 are used as the negative control. Twenty-four hours after infection, cells are washed with PBS and fixed with 4% formaldehyde in PBS (pH 7.4) at 4°C for 20min. ACE2 expression is detected by using goat anti-human ACE2 immunoglobulin followed by FITC-labelled donkey anti-goat immunoglobulin. Viral replication is detected by using rabbit antibody against the nucleocapsid protein of bat SARSr-CoV followed by Cy3-conjugated mouse anti-rabbit IgG. Quantitative real-time PCR and plaque assays will be conducted to determine the viral titers and growth kinetics in the infected cells at different time post-infection.

3.3.c Primary human airway epithelial (HAE) cultures: Primary HAE cultures represent highly differentiated cultures from the lungs of lung transplant recipients represent highly differentiated human airway epithelium containing ciliated and non-ciliated epithelial and goblet cells, grown on an air-liquid interface for several weeks prior to use (*18, 55, 56*). We will prepare HAE cultures from three different patient codes in triplicate in collaboration with UNC. Primary HAE cultures from three different patient codes will be inoculated with chimeric bat SARSr-CoVs to assess efficient replication. At 72 hpi, cultures will be fixed for immunofluorescent staining using antisera to detect SARSr-CoV conserved nucleocapsid protein (N) (*57, 58*). SARSr-CoVs that differ significantly in S protein sequence (11-25%) will be prioritized. For chimeras that replicate *in vitro*, we will use a panel of broadly cross-reactive mAbs to SARS-CoV. We will specifically use the SARS-CoV cross reactive human mAbs S227.14, S230.15, S109.8, S124.3, S230.15 and S227.14.

Progress report publication list: R01 AI110964, Daszak PI, Project Period: 06/01/2014 – 05/31/2019

The following are peer-reviewed papers published from work funded by the NIAID R01 during the project period (1-18). Other manuscripts on behavioral risk, phylogenetic and viral risk characterization are in prep and awaiting submission prior to the end date of the report.

1. Hu et al., Detection of diverse novel astroviruses from small mammals in China. *Journal of General Virology* **95**, 2442-2449 (2014). PMID: 25034867

2. B. Hu, X. Y. Ge, L. F. Wang, Z. L. Shi, Bat origin of human coronaviruses. *Virology journal* **12**, (2015). PMID: 26689940

3. V. D. Menachery et al., A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nature Medicine* **21**, 1508-+ (2015). PMID: 26552008

4. J. N. Mandl et al., Reservoir Host Immune Responses to Emerging Zoonotic Viruses. *Cell* **160**, 20-35 (2015). PMID: 25533784

5. M. N. Wang et al., Longitudinal surveillance of SARS-like coronaviruses in bats by quantitative real-time PCR. *Virologica Sinica* **31**, 78-80 (2016). PMID: 26920711

6. 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). PMID: 26807755

7. X. Y. Ge et al., Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft. *Virologica Sinica* **31**, 31-40 (2016). PMID: 26920708

8. L.-P. Zeng et al., [title obscured] Accessory Protein, ORFX, [text obscured] **90**, 6573-6582 (2016). PMID: 27170748

9. 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). PMID: 26719272

10. K. J. Olival et al., Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, (2017). PMID: 28636590

11. L. P. Zeng et al., Cross-neutralization of SARS coronavirus-specific antibodies against bat SARS-like coronaviruses. *Science China-Life Science* **60**, 1399-1402 (2017). PMID: 29134417

12. X. L. Yang et al., Genetically Diverse Filoviruses in Rousettus and Eonycteris spp. Bats, China, 2009 and 2015. *Emerging Infectious Diseases* **23**, 482-486 (2017). PMID: 28221123

13. 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). PMID: 29190287

14. N. Wang et al., Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China. *Virologica Sinica* (2018). PMID: [obscured]

15. 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). PMID: 29669833

16. Y. Luo et al., Longitudinal Surveillance of Betacoronaviruses [text obscured] During 2009-2016. *Virologica Sinica* **33**, 8[obscured]

17. Z. Wu et al., [text obscured] to better understand the wildlife origin of emerging infectious diseases. *[obscured]*

18. P. Zhou et al., Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature* **556**, 255-258 (2018). [obscured]

PHS Human Subjects and Clinical Trial

OMB Number:
Expiration Date: 03/31/2020

Are Human Subjects Involved? ● Yes ○ Yes ○ No

Is the Project Exempt from Federal regulations

Exemption Number ☐ 1 ☐ 2 ☐ 3 ☐ 4

Other Requested Information

Human Subjects Studies

Study#	Study Title	Clinical Trial?
1	Understanding the surveillance to captu... ...SrCoV spillover, routes of exposur...	

Section 1 - Basic Information (Study

Expiration Date: 03/31/2020

1.1. Study Title *

Understanding the Risk of Bat Coronavirus Emergence
SARSr-CoV s... routes of e...

1.2. Is this study ex... ...t from Fed...al
Regulations *

1.3. E...

1.4. Clinical Trial C... ...re *

    1.4.a. Does the ...

    1.4.b. A... participant...s prospectively assigned to an intervention?   No   ○ Yes

    1.4.c. Is ... ...ned to evaluate ... ...ffect of the ... ○ Yes   ● No
participants?

    1... ...Is the effect that ... eva... ○ Yes   ● No
behavioral ...utcome?

1.5. Provide the ClinicalTrials.gov Identifier (e.g.
NCT87654...