Modified Project Summary/Abstract Section

Enter the text here that is the new abstract information for your application. This section must be no longer than 30 lines of text.

Zoonotic coronaviruses (CoVs) are a significant threat to global health, as demonstrated by the emergence of SARS, MERS, and COVID-19. Our group identified bats as the wildlife reservoirs of SARS-CoV, and we have now published hundreds of novel SARS-related CoV (SARSr-CoV) sequences. Work under our previous NIH funding, and by others, has demonstrated that bats in Asia harbor an extraordinary diversity of SARSr-CoVs, some of which can use human ACE2 for cell entry, cause SARS-like illness in laboratory animals, and may evade current therapies or vaccines. Our analysis of ecological and human serological data indicates a median of around 66,000 people are infected by bat-SARSr-CoVs in the region each year, with unknown public health impacts. In this modification to our renewed R01, first awarded in 2019, we plan three aims**: Aim 1.** Analyze more than 300 full genomes/large genome segments of bat SARSr-CoVs from our prior bat sampling in southern China to identify viral characteristics, host biological traits, and environmental/ecological factors that lead to 'recombination hotspots'. We will use host range modeling, complex phylogenetics, and ecological, environmental and demographic data to assess factors that may enhance recombination. We will also analyze spillover risk from over 200 other bat CoV RdRp sequences identified in our prior work to assess the generality of correlates of SARSr-CoV spillover risk for all bat-CoVs; **Aim 2.** Use our community- and clinic-based survey data and archived pre-COVID-19 human samples to identify putative SARSr-CoV spillover events, routes of exposure, and potential public health consequences. We will test samples with a SARSr-CoV ACE2 surrogate virus neutralization to identify pre-COVID-19 infection with SARSr-CoVs and other ACE2 binding CoVs. We will test archived clinic-based syndromic surveillance samples to assess if patients presenting with influenza-like illness and severe acute respiratory illness have PCR or serological evidence of SARSr-CoV infection; **Aim 3.** Use computer modeling and cell culture to analyze potential binding interactions among novel bat-CoVs, putative reservoir and intermediate hosts, and people, to validate spillover predictions from Aims 1 & 2. Rather than use recombinant virus technology, we will estimate CoV binding to human cells using amino acid sequence analysis, modeling of RBD binding to human, bat & other putative host ACE2 and other receptors, and binding assays with non-infectious viral proteins and pseudovirus technology in vitro. We will then test our hypothesis that SARSr-CoVs with 10-25% spike protein sequence divergence from SARS-CoV or SARS-CoV-2 are able to infect human cells, and evade therapeutics and vaccines. Work from all three aims will help demonstrate proof-of-concept that our bat CoV program is a model platform to integrate analysis of virological and ecological factors contributing to CoV emergence and inform high impact strategies to prevent future pandemics. This includes providing critical reagents, therapeutic interventions, and viral genome sequences for pandemic and public health preparedness to counter future potential coronavirus outbreaks.

Modified Specific Aims Section

Enter the text here that is the new specific aims information for your application. One page is recommended.

Zoonotic coronaviruses represent a significant threat to global health, as demonstrated by the emergence of SARS-CoV, MERS-CoV and SARS-CoV-2. Our previous NIH-funded work identified bats as the wildlife reservoirs of SARS-CoV, and we have now published hundreds of novel SARS-related CoV (SARSr-CoV) sequences. We showed that bats in Asia harbor an extraordinary diversity of SARSr-CoVs, some of which are able to use human ACE2 for cell entry, cause SARS-like illness in laboratory animal models, and evade available therapies or vaccines. We used our human serological data to estimate that bat-SARSr-CoVs infect a median of around 66,000 people in the region, with significant potential for future public health impacts. Our renewed R01, awarded in 2019 but halted during Year 1, aimed to identify high zoonotic-risk bat-CoVs in China, identify community- and clinic-based evidence of their spillover, and characterize viruses to obtain data and reagents of value in surveillance, diagnostics and vaccine development.

In these modified specific aims, we have removed all on-the-ground work in China, all further field sampling of people or bats, and all recombinant virus culture or infection experiments. Work will now be conducted only

at EcoHealth Alliance, USA and at Duke-NUS, Singapore to: **1)** characterize and analyze >300 full genomes/large genome segments of SARSr-CoVs from our previous bat sampling in China and from other archived samples to determine the processes underlying CoV recombination and identify viral strains with high predicted risk of spillover; **2)** analyze archived samples from community- and clinic-based syndromic surveillance of people to identify evidence of spillover, and assess behavioral risk factors and evidence of illness; and **3)** conduct *in silico* and *in vitro* viral characterization and analysis of epidemiological data to identify hotspots of future CoV spillover risk. Testing of these archived samples is not budgeted on any other awards to EcoHealth Alliance. This work will follow 3 specific aims:

Aim 1: Identify high spillover-risk bat-SARSr-CoVs and assess drivers of recombination. We will analyze more than 300 full genomes/large genome segments of bat SARSr-CoVs sequenced from our prior bat sampling in China. At EcoHealth Alliance, we will conduct in-depth recombination analysis on these genomes and other genomes to identify viral characteristics, host biological traits, and environmental/ecological factors that correlate with recombination to identify 'recombination hotspots'. We will analyze the biological (e.g. host reservoir), and environmental (e.g. climate, landscape) factors that may enhance recombination. At Duke-NUS, we will test up to 1,000 archived bat samples per year using PCR and serology to identify which hosts carry SARSr-CoVs that are found to have spilled over in **Aim 2** and analyze them at EHA using the same phylogenetic, deep recombination analysis and ecological hotspot approach. Using archived samples from prior work, we will sequence ACE2 receptors of bat and putative intermediate hosts, and use these to assess likelihood of CoV spillover via intermediate hosts in **Aim 3**. At EcoHealth Alliance, we will also analyze host and environmental correlates of bat HKU-CoV diversity from >200 RdRp sequences identified in our prior work to assess the generality of correlates of SARSr-CoV spillover risk for all bat-CoVs.

Aim 2: Community- and clinic-based surveillance of archived pre-COVID-19 human samples to identify SARSr-CoV spillover events, routes of exposure, and potential public health consequences. We have >2,000 archived serum samples collected prior to the COVID-19 pandemic from communities located within SARSr-CoV spillover hotspots in SE Asia. These include people with high wildlife contact and those reporting respiratory disease. At Duke-NUS we will test these using our SARSr-CoV ACE2 surrogate virus neutralization test that can distinguish antibodies to multiple bat-CoV lineages, SARS-CoV, and all variants of SARS-CoV-2. We will access and test further archived sera (maximum 9,350) from our collaborative network in the region as the work proceeds. At EcoHealth Alliance, we will assess individual human seropositive status against data on human-wildlife contact and exposure risk collected in surveys and from biogeographic databases, and against environmental and demographic data. This includes as-yet-unanalyzed survey data from China and other SE Asia countries previously collected by our team. We will test archived clinic-based syndromic surveillance samples from SARSr-CoV hotspots to identify patients presenting with influenza-like illness and severe acute respiratory illness, and test samples for PCR- and serological evidence of SARSr-CoV infection.

Aim 3: Characterize SARSr-CoV binding, ability to evade therapeutics/vaccines, and identify spillover hotspots. We will use *in silico* and *in vitro* approaches to analyze potential host-virus interactions, assess ability to bind to human and other animal cells and validate spillover predictions from **Aims 1 & 2**. Rather than use recombinant virus technology, at EcoHealth Alliance we will estimate the ability of CoVs to bind to human ACE2 and other receptors using amino acid sequence analysis of binding affinity. At Duke-NUS, we will conduct computer modeling of RBD binding to ACE2 and other receptors of humans, bats & other putative hosts. We will conduct binding assays using non-infectious viral proteins *in vitro* and pseudovirus technology. All genome sequences, proteins, and assay results will be shared rapidly with the scientific research community to allow further characterization and diagnostic/countermeasure development work to proceed. We will test our hypothesis that SARSr-CoVs with 10-25% divergence in S protein sequences from SARS-CoV or SARS-CoV-2 are likely able to infect human cells, and to evade mAb therapeutics and vaccines. We will adapt our ACE-2 surrogate neutralization test to include bat and intermediate host ACE2 sequences to further assess likelihood of spillover. At EHA, we will refine spatial models of bat hosts and other ecological risk factors to identify the key 'hotspots' of risk for future spillover**.**

These modified specific aims remain true to the original goals of our renewed R01: characterizing the risk of CoV spillover. We aim to demonstrate proof-of-concept that our bat-CoV program is a model platform to integrate analysis of virological and ecological factors contributing to CoV emergence while informing high

impact strategies to prevent future pandemics. This includes providing critical reagents, therapeutic interventions, and viral genome sequences for future SARSr-CoV pandemic and public health preparedness.

Modified Public Health Relevance Section

Enter the text here that is the new public health relevance information for your application. Using no more than two or three sentences, describe the relevance of this research to public health.

Most emerging human viruses come from wildlife, and coronaviruses in particular represent a significant threat to public health and biosecurity in the US and globally, as was demonstrated by the SARS, MERS, and COVID-19 outbreaks. This project seeks to understand what factors allow bat-origin coronaviruses, including close relatives to SARS, to jump into the human population by studying their evolutionary diversity, the patterns of spillover in people that live in high-risk communities, and analyzing characteristics of bat coronaviruses that could allow them to emerge. This work will produce reagents and genetic sequences that can be used to test vaccines and therapeutics to fight future pandemics, and hotspot maps that can be used to target surveillance and control measures to prevent their emergence.