VERTEBRATE ANIMALS

1. Detailed description of animal use.

Work with vertebrate animals will be conducted in Thailand and Malaysia for field sampling (collection of diagnostic clinical specimens) of free-ranging mammals; and the University of North Carolina, Chapel Hill, USA and the National Emerging Infectious Diseases Laboratory (NEIDL), Boston, USA for mouse model experimental infections.

Wild animal captures:

Capture and sampling techniques for all wild animals (bats, rodents and non-human primates) 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, Phelps, 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 a draft IACUC application for this proposed project, and will submit it within 2 month of the project's start date (to Tufts University) to minimize delays in beginning Year 1 field sampling.

Bats: 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-Is 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 in a cool place until samples are collected. Bats are held for a maximum of six hours (typically less than 3 hours), and released after sampling.

Rodents: Free-ranging rodents will be captured through pit traps and box traps. Food, water and shelter will be provided. Traps will be checked a minimum of twice daily, in the morning and in the afternoon. If adverse weather (extreme heat, rain) is expected or researchers are working in areas where predation is common, traps will be checked more frequently, and closed during the adverse weather. Padding, shelter and food and water will be provided in traps.

Non-human primates: Free-ranging and captive non-human primates will be chemically restrained (by darting with anesthetic or through manual chemical injection), and handled only for the duration of sampling. Where possible, small primates (<15 kg) will be captured with nets, using a hoop and mesh size appropriate to the size of the animal. Alternatively, medium sized primates (e.g. free-ranging macaques) will be captured using metal traps placed on flat ground in a secure area or on a pallet constructed on a tree. Trapped animals will be transferred to a transfer cage placed against a sliding door and covered.

Sample Collection from wild animals:

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 smaller insectivorous bats (<50g) using a 27g needle to puncture the brachial artery and a 70ul hematocrit tube to collect the blood. For larger bats (>50g) we will collect blood from either the cephalic vein or from the radial artery or vein using a 25 or 23 gauge needle and 3cc syringe.

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).

Non-human primates: Chemical immobilization of any small primate species will be preceded by accurate weight determination. For anesthesia, ketamine (8-15 mg/kg depending on animal size) or a combination of tiletamine/zolazepam (7-8mg/kg in smaller animals; 4-6mg/kg in medium sized animals; 3-4mg/kg in larger animals; and 2- 3mg/kg in great apes) or ketamine/medetomidine (3.5 mg/kg ketamine and 0.035 mg/kg of medetomidine), or ketamine/xylazine (100mg/ml each) in a 1:1 ratio at a dose of 10mg/kg IM will be administered, using low doses where possible to allow for relatively rapid sampling. Caution will be taken when administering chemical immobilizing drugs to animals in estrus to prevent against hemorrhage of the tumescent area. Immobilized animals will be allowed to recover in isolation. Sampling procedures for non-human primates will include venipuncture; fecal, urine, and external parasite collection; biopsy of lesions and/or skin samples for genetic testing; oral, nasal, urogenital and anal swabs, plucked hair and milk if/when available. Blood samples from larger primate species will primarily be collected from the forearm veins (cephalic, radial, median, and ulnar veins). In smaller species, the femoral or caudal saphenous veins will be preferred. In very small non-human primates the jugular vein may be the only option. Following handling and when appropriate, benzodiazepine and alfa-2 agonist sedation will be chemically reversed, and the animal returned to its group of con-specifics in the immediate vicinity of their capture, with appropriate observation.

Laboratory animals (mouse models):

The goal of these studies is to identify if novel high risk emerging coronaviruses (CoV), filoviruses (FVs) and henipaviruses that circulate in bats and/or other reservoir species are poised for emergence in human populations, and to assess pathogenicity for selected strains of CoVs, FVs, and henipaviruses. High containment BSL3 and BSL4 pathogens will be recovered from natural infections in reservoir species and/or humans, or recovered from whole genome sequences by reverse genetics using synthetically derived molecular DNA clones. Viruses will be used to infect classic laboratory mouse strains (eg, C57BL6/J), transgenic mice expressing human entry receptors for different coronaviruses, filoviruses or henipaviruses, the Collaborative Cross Genetic Reference populations, or mice reconstituted with the bat immune system designed to identify new mouse models of human disease.

Animal experiments with mice will be performed at the University of North Carolina (UNC), Boston National Emerging Infectious Diseases Laboratory (NEIDL) in dedicated facilities under the direction of the research PIs. Prior to infection studies, the animals will be maintained in Sealsafe™ HEPA-filtered air in/out unit or compatible systems for at least one week prior to virus challenge. In addition, laboratory personnel inspect animals daily and any animal in distress is immediately euthanized (moribund, unresponsive, loss of more than approved percentage of starting weight). Animal care and housing follows IACUC recommendations and all personnel have attended mandatory IACUC training courses. A trained veterinarian is always on call to assist with animal care and husbandry. Below we summarize the description of procedures for each specific Aim, justifications, minimization and pain and distress and methods for euthanasia.

Mice

Species: *Mus musculus*; CC mouse lines and knockout mice

Ages: Adults 6 to 8 weeks of age and C57BL6 mice of 1.5 years of age

Sex: Females and males

Procedures for laboratory animals:

Animal work will be done at the above-mentioned facilities in accordance with the Guide for the Care and Use of Laboratory Animals. The minimum numbers of animals will be used in order to achieve our experimental goals with statistical significance.

Specific Aim 1: Identify, characterize and rank spillover risk of high zoonotic potential viruses from wildlife.

Rationale: The goal of these studies are to use mouse models to assess human disease for novel viruses discoved in wildlife populations. We note that many zoonotic viruses replicate poorly in rodents, requiring adaptation. To circumvent this problem, we will use genetically distinct mouse resources (CC mice), mouse strains expressing human entry receptors and standard laboratory mice to identify mouse models for studying viral pathogenesis, as well as to evaluate vaccine and antiviral treatment regiments.

These studies require tractable experimental model organisms like the CC with sets of defined genetic characteristics (enumerated as follows). Genetically defined and immortal reference populations that allow whole-organism study of natural variation is a powerful approach for modeling human disease susceptibility and cannot be achieved by means other than the use of whole animal genetics. Mice are excellent model systems to study human gene function for the following reasons:

a. The genome has been sequenced and there are many genetic tools available for follow-up and complementary studies.
b. Mice share many aspects of anatomy, physiology, patterns of development, and control of gene expression with humans.
c. The Collaborative Cross (CC) is a large panel of recombinant inbred strains that have been specifically bred to randomize an extraordinarily large amount of genetic variants and has been designed to be an optimal model of the human heterogeneous population while allowing further probing into tissue and cell-type specific responses
d. The CC support integration of data across time, location, demography, and environments.
e. Reference populations of mice provide suitable whole-organism models for human viral infection responses, which to date cannot be replicated faithfully by *in vitro* models.
f. The resource is portable and available worldwide.

Experimental Design and Sample Sizes:

Experiment 1
Mice
Twenty mice (10 males and 10 females) will be infected with six different test viruses/yr.

20 mice x 6 viruses = 120 mice
Total number of mice= 120 mice

Total Animals	# Pain Category C	# Pain Category E
120	100	20

Experiment 2.
Humanized Mice
C57BL6/J-hACE2 Mice
C57BL6/J-hDPP4 288/330 Mice

10 mice (5 female and 5 male) x 4 SARS-like coronaviruses x 2 repeats= 80 mice
10 mice (5 female and 5 male) x 4 MERS-like coronaviruses x 2 repeats= 80 mice
Total numbers of animals for aim 1/yr= 160 mice
Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
1200	1080	120

Experiment 3
Mice
Six mice (3 males and 3 females) from 6 Collaborative Cross mouse lines (6 to 8 weeks of age) will be infected with six test viruses/yr.

6 mice x 6 lines x 6 viruses= 216 mice
12 mice x 2 lines x 1 virus= 24 mice
Total number of mice for Aim 1 screening/yr: 240 x 5 years= 1200 mice

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
1200	1080	120

We note that wild-type EBOV, MARV, NiV, HeV and SARS-CoV causes subclinical infections in standard laboratory strain mice.

Specific Aim 2 and 3. Discovery of Novel Viruses from Human Source Samples. We anticipate discovering one novel virus in focal high-risk human populations/yr.

Rationale: The goal of these studies are to identify novel mouse models of human disease. We note that newly discovered human viruses replicate poorly in rodents, requiring adaptation. To circumvent this problem, we will use genetically distinct mouse resources (CC mice), mouse strains expressing human entry receptors and standard laboratory mice to identify mouse models for studying viral pathogenesis, as well as to evaluate vaccine and antiviral treatment regiments.

Experiment 1
Mice
Twenty mice (10 males and 10 females) will be infected with one different test viruses/yr.

20 mice x 1 viruses x 2 repeats = 40 mice
Total number of mice = 40 mice x 5 years = 200 mice

Total Animals	# Pain Category C	# Pain Category E
200	160	40

Experiment 2.
Humanized Mice
C57BL6/J-hACE2 Mice
C57BL6/J-hDPP4 288/330 Mice

20 hACE2 mice (10 female and 10 male) x 1 SARS-like coronaviruses x 2 repeats= 40 mice
20 hDPP4 mice (10 female and 10 male) x 1 MERS-like coronaviruses x 2 repeats= 40 mice
Total numbers of animals for aim 1/yr= 80 mice x 5 years = 400 mice
Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
400	300	100

Experiment 3
Mice
Six mice (3 males and 3 females) from 8 Collaborative Cross mouse lines (6 to 8 weeks of age) will be infected with one test viruses/yr.

6 mice x 8 lines = 48 mice
16 mice x 2 lines = 32 mice
Total number of mice for Aim 1 screening/yr: 80 mice x 5 years= 400 mice

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
400	320	80

We note that wild-type EBOV, MARV, NiV, HeV and SARS-CoV CoV cause subclinical infections in standard laboratory strain mice. It is anticipated that disease severity will increase in select CC mouse strains.

2. **Justify use of animals, choice of species, numbers to be used**.
Wild animals: Species and number used in study: The purpose of this study is to conduct analyses to geographically- and taxonomically-target testing of samples from wild mammals that are most likely to harbor known high-profile zoonotic pathogens, or close relatives with potential to infect people. We will analyze archived specimens and only collect additional specimens projected from each prioritized taxa to achieve statistically robust sample sizes and to complement our archived specimens. Preliminary analysis indicates that, for all viral target families, we will require ~14,000 new specimens from bats (equating to no more than 7000 individual animals) to identify 80% of remaining bat viral species diversity in our study region, ~5,000 samples from Thailand and ~9,000 samples from Malaysia. Viral strain diversity from a sampling effort this size is expected to number in the hundreds of strains. For example, given ~6% prevalence of CoVs in bat species previously sampled under the PREDICT project, a target of 14,000 individuals would yield ~800 PCR positive individuals, representing, an estimated ~600 novel sequences/strains. Similarly, for PMVs, which have an average PCR detection prevalence of ~1.5%, sampling of 14,000 individuals would yield ~200 positives and an estimated 150 novel strains. The required numbers of specimens to capture 80% of CoV and PMV viral diversity in free-ranging rodents and NHP will be approximately half that of bats given lower levels of viral diversity we expect to capture, for a total of no more than 7,000 specimens (equating to no more than 2300 individuals). Filovirus estimates are not feasible yet due to the small number of positive samples in prior studies, but will be estimated as the project begins.

Laboratory animals: This proposal aims to develop novel mouse models that display human disease phenotypes following infection with phylogenetically unique zoonotic and human strains of these viruses. These studies cannot be done without vertebrate animals. There is no *in vitro* system that accurately mimics virulence of highly pathogenic viruses, or be able to predict disease outcomes following infection. At this time, there is no substitute for *in vivo* efficacy studies. Our studies are designed with the fewest number of animals while retaining statistical significance. The numbers of animals proposed in this study will provide robust statistical confidence of our results.

The Collaborative Cross mice are genetically distinct so infection of these mouse strains can result in wildly different clinical disease outcomes. As such, the CC model will identify unique lines that accurately recapitulate many aspects of the human disease phenotypes and will provide essential information for the testing of vaccines and drugs against these highly pathogenic RNA viruses.

3. **Provide information on veterinary care**. For wild caught animals, veterinary oversight will be provide by EcoHealth Alliance's wildlife veterinarians, led by Senior Veterinarian, Dr. Field. 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.

The UNC and NEIDL have veterinary staff and team of experienced animal care technicians who will oversee the housing and care of bats used for experimental infections in their facilities.

4. **Procedures for ensuring animal comfort, lack of distress, pain, or injury**:
Wild animals: Wild animals will not be held longer than 6 hours during the sampling process. Co-Is Olival and Phelps and Dr. Field from EHA, and co-Is Wacharapluesadee and Hughes (from Thailand and Malaysia, respectively) have extensive experience in capture, anesthesia, and sampling wildlife, especially bats and will lead oversight and training of field teams. In our team's experience, wild animals 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. Rodent traps will be checked a minimum of twice daily, in the morning and in the afternoon. If adverse weather (extreme heat, rain) is expected or researchers are working in areas where predation is common, traps will be checked more frequently, and closed during the adverse weather. Padding, shelter and food and water will be provided in rodent traps. 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.

Laboratory animals: Mouse adapted strains of SARS- and MERS-CoV replicate efficiently in the lungs of mice and may produce significant disease in young and aged animals, including acute onset respiratory distress syndrome, a clinically devastating end stage lung disease with 50% mortality rates. It is unclear whether newly discovered SARS-like or MERS-like coronaviruses or other bat viruses will cause severe disease in mice. Mice will be closely monitored daily for signs of clinical disease. Since analgesics may affect the outcome of infections, analgesics will not be used and we will rely on close monitoring and euthanasia of sick animals to prevent undue pain and suffering. In general, animals will be euthanized if they approach losing >25% of their starting weight; we recognize that this is a significant weight loss, but it is acceptable in these studies as some animals can recover from >25% weight loss after highly pathogenic coronavirus infection. We will euthanize moribund animals, regardless of weight loss criteria. Euthanasia will be performed by overdose with isoflurane. This will immediately be followed by organ harvest/exsanguinations, as prior treatment with these agents ensure that the animals will not suffer during this procedure due to operator error. This approach was chosen because unconsciousness and death occur quickly and the method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

5. Euthanasia

Wild animals: In the event of injury to a wild animal in the field 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 or intraperitoneally. This protocol is in accordance with the AVMA euthanasia guidelines (2013). 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.

Criteria for euthanasia for mice: Mice will be euthanized (isoflurane overdose followed by organ harvest) at the point at which they become moribund, lose over 20 percent of their starting weight (or up to 30 percent of their starting weight if the animals are being maintained under the weight loss exception), reach a clinical score of 4 or higher or reach pre-determined endpoints (ranging from 1 to 7 days post infection), whichever comes first.

0. No clinical signs
1. Ruffled fur.
2. Ruffled fur with hunched posture (only slight or no signs of dehydration)
3. As above with more severe signs of dehydration and some loss of body strength, some loss of spontaneous morbidity
4. Marked loss of spontaneous morbidity and pronounced dehydration
5. Moribund: unresponsive to stimuli and pronounced eye squint

-All scores except 5 can be qualified with a 0.5 for severity
-Any mouse that reaches a clinical score of 4 or more will be euthanized immediately

Criteria for euthanasia for bats: Bats will be euthanized (isoflurane overdose followed by organ harvest) at the point at which they become moribund, lose over 20 percent of their starting weight, reach a clinical score of 4 or higher or reach pre-determined endpoints (ranging from 1 to 7 days post infection), whichever comes first.

0. No clinical signs
1. Decreased activity, behavioral abnormalities.
2. Difficulty flying (only slight or no signs of dehydration)

3. As above with more severe signs of dehydration and some loss of body strength, some loss of spontaneous morbidity.
4. Marked loss of spontaneous morbidity and pronounced dehydration.
5. Moribund: unresponsive to stimuli and pronounced eye squint.
-All scores except 5 can be qualified with a 0.5 for severity.
-Any bat that reaches a clinical score of 4 or more will be euthanized immediately

SELECT AGENT RESEARCH/BIOHAZARDS. Yes

Select Agent Research:

Research on Biological Select Agents and Toxins (BSAT) requires strict compliance with regulations that are primarily overseen by the US Centers for Disease Control and Prevention (CDC) and the Animal and Plant Health Inspection Service (APHIS). These regulations are designed to maintain the safety and security of the materials, personnel and the environment. Per requirements, we outline details of select agent research below for our two EID-SEARCH core laboratory partners, The University of North Carolina at Chapel Hill (UNC) and National Emerging Infectious Diseases Laboratories (NEIDL), who will conduct select agent research.

Identify the Select Agent(s) to be used in the proposed research:

SARS-CoV: We propose using Severe Acute Respiratory Syndrome-associated Coronavirus (SARS-CoV) and SARS-CoV genome RNA (isolated using TriZOL) in this proposal. Derivative viruses encoding 2/3 genome length SARS-CoV are also considered as select agents; consequently, recombinant SARS-CoV encoding various bat SARS-like S glycoproteins will be considered select agents.

Henipaviruses: Wildtype Nipah, Hendra and related bat viruses are select agents and will be used at the Boston NEIDL BSL-4 Laboratories. Nipah virus is an overlap select agent regulated by both HHS and USDA.

Filoviruses: Wildtype Ebola viruses are select agents and will be used at the Boston NEIDL BSL-4 Laboratories.

Provide the registration status of all entities where Select Agent(s) will be used:

The UNC 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.

The NEIDL Environmental Health and Safety (EHS) group to vet, review and approve Standard Operating Procedures, consistent with BU-wide policies and procedures, which meet and/or exceed all applicable federal, state, and local regulations (NIH, BMBL, OSHA, CDC, NRC, MWRA, DEP, BFD, etc.). EHS responsibilities include: biosafety; laboratory and building occupant safety; wastewater management and monitoring; hazardous and non-hazardous solid waste management; biological, chemical, and radioactive waste disposal management; select agent program, medical surveillance program, staff and first responder training programs. For each NEIDL research project which has been approved by the BU Institutional Biosafety Committee (IBC), the EHS Biosafety Manager obtains applicable select agent permits to comply with 7CFR331 and 9CFR121, the Agricultural Bioterrorism Protection act of 2002; *Possession, Use, Transfer of Biological Agents and Toxins: Final Rule*. If an agent-specific immunization is available the EHS Core Biosafety Manager will assist the Research Occupational Health Program physicians to determine whether laboratory personnel will require immunizations for agents handled or present in the laboratory.

Provide a description of all facilities where the Select Agent(s) will be used:

UNC: SARS-CoV and related derivative viruses will be manipulated in research activities (UNC) including establishing growth curves and performing plaque assays in laboratory spaces that meet the 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 the BL-3 criteria outlined in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (March 2013). In addition, all mouse work at the University of North Carolina at Chapel Hill will be performed in the approved and registered BSL-3 lab, equipped with Techniplast Sealsafe™ Hepa-filtered animal housing for rodents. All protocols will be approved by the IACUC at the University of North Carolina at Chapel.

At UNC, all 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, as well as mechanical system monitors and alarms to support effective isolation and containment of operations

involving SARS-CoV. The anterooms to the BSL-3 labs house PAPR charging stations, lab and safety supplies, and a changing area. For both the BSL2 and BSL3 select agent spaces, access to the select agents is restricted by the hallway to anteroom door, which requires swipe card and punch codes for entry, the door between the anteroom and the BSL-3 (BL3 only), and freezer locks.

NEIDL: The select agent work to be carried out will be performed in the National Emerging Infectious Diseases Laboratories (NEIDL). The NEIDL is fully permitted for Select Agent work at BSL-3 and ABSL-3, as well as BSL-4 and ABSL-4. We will conduct experimental infection studies using Nipah and Hendra (Henipaviruses) and Ebola and Ebola-related viruses at NEIDL using animal models and cell lines within the biosafety level 4 laboratory at NEIDL. No infectious materials will be moved or handled outside of the BSL 4 facility. All science and animal care staff are fully trained and certified to work under BSL 4 conditions.

At Boston University (BU), research with BSAT is focused to combat infectious diseases caused by the agents, with primary concentration on the development of vaccines, therapeutics and diagnostic tests. Work is conducted in highly specialized containment facilities that are specifically designed, verified and maintained to ensure that the materials are secured, contained and handled safely. Research facilities with BSAT at BU have received prior approval from CDC/USDA and other local regulatory agencies prior to the possession, storage and conducting of research work. All BSAT research proposals are first carefully reviewed by the Institutional Biosafety Committee (IBC) and, together with the Environmental Health and Safety (EHS), checks and verifies that the required safety procedures, personal protective equipment, safety equipment, and trainings are in place and implemented prior to initiation of research work.

A senior Scientific Safety Officer serves as the Select Agent Responsible Official (RO) and has oversight for the implementation of the BSAT program. Under this program, the institution implements and maintains a system of control measures to ensure that all personnel are properly screened for security risks and have passed the required background verification checks by Federal agencies and BU. Personnel complete and meet the medical and health screenings required by the Research Occupational Health Program (ROHP); personnel are appropriately trained on the hazards of the materials, safety procedures and their roles and responsibilities. The RO and the Alternate Responsible Officials (ARO) conduct an annual review of the Security, Biosafety and Incident Response Plans and make necessary changes to the plans.

Describe the procedures that will be used to monitor possession, use and transfer of the Select Agent(s): 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 lab, documenting lab 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 Lab Manager and Responsible Official in accordance with standard operating procedure, including obtaining appropriate permits for shipping select agent materials. Lab managers of both facilities are DOT/IATA trained for shipping dangerous goods and will follow all regulations for shipping, both under dangerous goods and select agent regulations. Transfer of select agent RNA in trizol from the BSL3 and BSL2 laboratory is witnessed by UNC and Vanderbilt ARO in a process that has been approved by the CDC.

For NEIDL, all biohazards that are shipped or received for approved projects are mandated to meet the standards of the High Hazard Materials Management policy, which states that BU will meet or exceed all applicable shipping regulations under the requirements of the U.S. Department of Transportation (DOT) and the International Air Transportation Authority (IATA). The RO, the BU Office of Emergency Management and Public Safety have responsibility for overseeing the transportation process for select agents and for contracting appropriate transportation vendors which utilize screened personnel, GPS tracking systems and can provide an all-inclusive chain of custody documentation for each shipment.

Describe plans for appropriate biosafety, biocontainment, and security of the Select Agent(s): Co-I Baric's

laboratories have been operational since 2004 with BSL-3 Core Policies and Procedures documentation in place with lab-specific standard operating procedures. BSL-3 standard operating procedures have been reviewed and approved by the Institutional Biosafety Committees at either the University of North Carolina at Chapel Hill or Vanderbilt and are updated as lab processes change or biosafety procedures evolve. The content of these documents has been reformatted and expanded to conform to the select agent regulations for the biosafety, security and incident response plans. Additionally, lab-specific security risk assessments have been completed and recommendations put in place to ensure that security measures and procedures are sufficient to effectively minimize the possibility of unauthorized access to select agent-regulated materials. Equipment and procedures will be modified if deemed necessary based on this assessment. The facilities at the University of North Carolina at Chapel Hill and Vanderbilt have undergone multiple CDC inspections and are currently in compliance with CDC requirements relating to SARS-CoV and select agent status.

Co-Is Keusch and Corley from NEIDL have endorsed a “safety first” environment which underpin all activities. The NEIDL Public Safety core is responsible for 24 hours a day, 7 days a week facility security and both police-academy highly trained public safety officers and specialized automated building systems are used. Public Safety personnel are well trained in the intricacies of a secure site maintenance, criminal applications with a significant amount of training pertaining to safety, facilities, emergency preparedness and response, biosafety incidents, animal rights activism, insider threats and coordinated notification and response of external local, state and federal responders. Individuals are only granted access to relevant NEIDL facilities based upon their responsibilities in the building. Employees pass through security layers that require identification by a public safety officer, and use a combination of proximity card access and biometric iris readers to gain access to laboratories in which select agent work is carried out. Activities in select agent laboratories can be monitored by closed circuit television, and video recordings can be reviewed. Access is monitored for compliance both electronically and by public safety staff. Variances to authorized access results in notifications of public safety staff within the building which will initiate a response to that variance.

Describe the biocontainment resources available at all performance sites: All BSL-3 and BSL-4 labs are under negative pressure, with redundant systems to ensure negative pressure is maintained. Both facilities have autoclaves to decontaminate all waste materials as well as approved protocols for the treatment or inactivation of any materials leaving the lab. All personnel are extensively trained in basic virology and safety protocols before being approved for select agent work and then undergo extensive training to work with SARS-CoV as a BSL-3 pathogen. In both labs, annual testing is performed to verify that the biosafety cabinets, lab supply/exhaust systems (including alarms), and other laboratory equipment are functioning as designed. The lab is 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.

OTHER BIOHAZADS

Other Biohazards and Non-Select BSL3 Agents: We will synthesize full length recombinant viruses for MERS-CoV and from a variety of SARS-like, MERS-like and related emerging coronaviruses (e.g., SADS-CoV, HKU2, HKU10, etc.). All of these viruses will be isolated and studied under BSL3 conditions at UNC.

Field Work Biosafety:

Introduction and Background. Many of the novel viruses studied in this proposal have caused or have the potential to cause human outbreaks with significant case fatality rates, and there are no vaccines available for many of these agents. The work proposed in this application will involve two aspects: field work and laboratory work, focusing on distantly coronaviruses, filoviruses and henipaviruses. Fieldwork involves the highest risk of exposure to bat and related human viruses of high pathogenic potential, 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, rodents, primates 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-like CoV, MERS-like CoV etc.), 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 and practice proper environmental disinfection techniques. This includes wearing coveralls or dedicated clothing, nitrile gloves, eye protection, and a P95 or P100 respirator, or positive air pressure respirators (PAPRs) in the field. All field clothing and equipment will be disinfected using Virkon disinfectant, and Tyvek suits will be properly disposed of as biohazard. 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 booster 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.

Available Treatments: No approved treatments are related for MERS-CoV, MERS-like bat CoV, SARS and the SARSr-related and other bat coronavirus infections, however, GS5734 is effective against most coronaviruses, filoviruses and henipaviruses. In addition, therapeutic human antibodies are available against many of the classic high path viruses studied in this proposal, although it is less certain whether these immunotherapeutics will be effective against more phylogenetically distant strains in the family. VSV-EBOV vaccine is used to prevent Ebola infections in humans, having been administered to over 130,000 humans, mostly in the DRC.

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.

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CONSORTIUM/CONTRACTUAL ARRANGEMENTS:

This project is a multi-institutional collaboration led by EcoHealth Alliance, New York (Daszak, PI), which will subcontract funds to five institutions: Chulalongkorn University's Thai Red Cross Emerging Infectious Disease Research Center (co-I Wacharapluesadee), Conservation Medicine Malaysia (co-I Hughes), the University of North Carolina at Chapel Hill (co-I Baric), the Uniformed Services University (co-I Broder), and Duke-NUS Medical School (co-I Wang). In addition, co-Is Keusch and Corley from Boston University's National Emerging Infectious Diseases Laboratories will provide additional in kind support, using additional funding, to attempt viral isolation on any novel Filoviruses or Henipaviruses we discover over the course of our award. PI Daszak has over 20 years previous experience managing collaborative projects including two R01s on Nipah virus ecology and an 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, and has conducted international, multi-disciplinary and multi-partner research around the world for more than 30 years. The subcontract institutions will work on specific issues and areas in which they have proven expertise. These areas are:

- Wildlife and human community surveillance and specimen collection, human clinical or hospital syndromic surveillance, screening and sequencing of specimens using conserved PCR assays for CoV, FV, and PMVs, screening of serum specimens using MMIA (Luminex) assays. (Chulalongkorn University TRC-EID, co-I Wacharapluesadee) and (Conservation Medicine Malaysia, co-I Hughes)
- Novel serological and molecular assay development; generation of reagents for novel assays; and training of Thailand and Malaysia laboratory staff for technology transfer for serological and molecular protocol development (Uniformed Services University, co-I Broder) and (Duke-NUS Medical School, co-I Wang).
- Small animal models of viral pathogenesis, primary human cell cultures, viral isolation and reverse genetics (University of North Carolina at Chapel Hill, co-I Baric) and (National Emerging Infectious Diseases Laboratories, co-I Keusch)

EcoHealth Alliance (EHA) led by PI Daszak have collaborated with all partners in the EIDRC consortium for 5-20 yrs on NIAID- and USAID-funded research, including more than 10 years each with co-Is Wacharapluesadee, Hughes, Baric, Broder, and Wang.

UNC
GILLINGS SCHOOL OF
GLOBAL PUBLIC HEALTH

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

DEPARTMENT OF EPIDEMIOLOGY
McGAVRAN-GREENBERG HALL
CAMPUS BOX 7435
CHAPEL HILL, NC 27599-7435

June 18, 2019

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

Reference: Response to RFA-AI-19-028 for grant entitled *Understanding Risk of Zoonotic Virus Emergence in EID Hotspots of Southeast Asia.*

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 March 1, 2020 to February 28, 2025. The work to be performed by UNC-CH does not include human research subjects but does include animal 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.

The principal investigator at EcoHealth Alliance is Dr. Peter Daszak and he will be collaborating with UNC-CH's Principal Investigator, Dr. Ralph Baric . The UNC-CH budget, budget justification and scope of work are provided as separate enclosures to this letter. The total cost of the proposed subcontract for all five years will not exceed $971,975 and this 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 let us know.

Sincerely,

Terry Magnuson, PhD
Vice Chancellor for Research
Email: (b) (6)

Ralph S Baric, PhD
UNC-CH Principal Investigator
Email: (b) (6)

06 June 2019

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

Dear Dr. Daszak,

This letter expresses the strong interest that staff at Duke-NUS Medical School has in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

The Duke-NUS Medical School recognizes the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

We at Duke-NUS have collaborated successfully with all partners on this proposal, and look forward to continuing our work together. Our work will include managing the test development and sample testing component of this multi-disciplinary project. We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Sincerely,

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

8 College Road, Singapore 169857
T +65 6516 7666 F +65 6221 7396
www.duke-nus.edu.sg
A school of the National University of Singapore (RCB No: 200604346E)

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799
www.usuhs.edu

Christopher C. Broder, Ph.D.
Professor and Chair

Tele: 301-295-3401 / Fax: 301-295-3773
E-mail: Christopher.broder@usuhs.edu

June 12, 2019

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

Dear Dr. Daszak,

This letter expresses the high interest that I and my laboratory have in continuing and expanding our collaborative research endeavors with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

I and the Uniformed Services University (USU) recognize the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value regionally and globally, by identifying key pandemic threats in an EID hotspot region.

My group at USU has collaborated successfully with all partners on this proposal, and look forward to continuing our work together. Our work will include the serological testing of samples in collaboration with partner laboratories, and we will actively take part in technology transfer and research cross-training in which we are well-experienced. We are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC. I look forward to this exciting opportunity to this network in place under the leadership of EcoHealth Alliance!

Sincerely,

Christopher C. Broder, Ph.D.
Professor and Chair
Department of Microbiology and Immunology

Boston University National Emerging Infectious Diseases Laboratories

620 Albany Street
Boston, Massachusetts 02118
bu.edu/neidl

/NEIDL

BOSTON UNIVERSITY

06 June 2019

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

Dear Dr. Daszak:

This letter expresses the high interest that faculty and staff at Boston University's National Emerging Infectious Diseases Laboratories (NEIDL) have in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

The NEIDL recognizes the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spill over into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

We at NEIDL have collaborated successfully with all partners on this proposal, and look forward to continuing our work together. Going from "sequences" to "viruses" is a critical unmet need in surveillance and in assessing spillover potential and we are happy to participate. We are particularly excited about the opportunities to have access to sequences of novel viruses that may need to have 3' or 5' ends "completed", as well as to assess the presence of viruses in samples that are PCR positive for isolation and culture under BSL-4 conditions. We will seek external funding to test these for infectivity in human cells, and make these available to other investigators in the USA. We have a number of investigators that are experienced working with pathogens at maximum containment, have the ability to test viruses for infectivity in a variety of human cell types, and to assess the uptake and receptor usage in these cells. We are already developing small molecule and monoclonal therapeutics with commercial partners, and also hope to receive convalescent sera, PBMCs and other samples from clinical cohorts to further these goals. Finally, we will actively take part in technology transfer and research cross-training with other partners, including externally-funded visiting scholars and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDRC CC.

Sincerely,

Ronald B. Corley, Ph.D.
Professor and Chair,
Microbiology Director, NEIDL

Gerald T. Keusch, MD
Professor, Medicine and International Health
Boston University Schools of Medicine and Public Health Associate Director, NEIDL

Thai Red Cross Emerging Infectious Diseases Health Science Centre (TRC-EID)
WHO Collaborating Centre for Research and Training on Viral Zoonoses

13 June 2019

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

Dear Dr. Daszak,

This letter expresses the high interest that staff at Thai Red Cross Emerging Infectious Diseases Health Science Centre (TRC-EID) has in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

The TRC-EID recognizes the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

We at TRC-EID have collaborated successfully with all partners on this proposal, and look forward to continuing our work together. Our work will include managing human surveillance, sample collection, testing, and development of laboratory diagnostics. We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Sincerely,

Thiravat Hemachudha

Prof. Thiravat Hemachudha
Director of
Thai Red Cross Emerging Infectious Diseases Health Science Centre and
WHO Collaborating Centre for Research and Training on Viral Zoonoses
Faculty of Medicine, Chulalongkorn University
(b) (6)

Conservation Medicine Ltd.
13H Villamas Condo,
Villamas, Jln Villamas
Off Jalan Sierramas Barat,
Sg Buloh, 47000,
Selangor, Malaysia.

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

06 June 2019

Dear Dr. Daszak,

This letter expresses the high interest that staff at Conservation Medicine Ltd have in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

I have worked with EcoHealth Alliance as the Project Coordinator for the "Emerging Pandemic Threat PREDICT Program" since 2010, as the Deputy Chief of Party on the USAID funded "Infectious Disease Emergence and Economics of Altered Landscapes" project since 2013 and as Co-PI on the DTRA funded Serological Biosurveillance for Spillover of Henipaviruses and Filoviruses at Agricultural and Hunting Human- Animal Interfaces in Peninsular Malaysia since May 2017. It is with great pleasure that I fully endorse your project and see high value in this work.

Conservation Medicine Ltd recognizes the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

We at Conservation Medicine Ltd have collaborated successfully with all partners on this proposal, and look forward to continuing our work together. Our work will include human and wildlife sample collection, coordination of syndromic surveillance and outbreak response, storage of samples and coordination of all in-country testing, and development of laboratory diagnostics. We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

We look forward to collaborating with you and EcoHealth Alliance on this worthwhile project.

Sincerely,

Tom Hughes
Director
Conservation Medicine, Ltd.
(b) (6)
(b) (6) (Mobile)
(b) (6) (Telephone/Fax)

06 June 2019

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

Dear Dr. Daszak,

This letter expresses the high interest that staff at Lintang Health Clinic, Sungai Siput, Kuala Kangsar District Health Office has in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

The Lintang Health Clinic, Sungai Siput, Kuala Kangsar District Health Office recognizes the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

We at Lintang Health Clinic, Sungai Siput, Kuala Kangsar District Health Office have collaborated successfully with all partners on this proposal, and look forward to continuing our work together. Our work will include managing human surveillance and sample collection. We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Sincerely,

Dr Jayaseelan Sekaran
Senior Medical Officer of Lintang Health Clinic,
Kuala Kangsar District Health Office,
33000 Kuala Kangsar,
Perak,
Malaysia
Email: (b) (6)

06 June 2019

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

Dear Dr. Daszak,

This letter expresses the high interest that staff at Pos Betau Health Clinic, Kuala Lipis District Health Office has in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

Pos Betau Health Clinic, Kuala Lipis District Health Office recognizes the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

We at Pos Betau Health Clinic, Kuala Lipis District Health Office have collaborated successfully with all partners on this proposal, and look forward to continuing our work together. Our work will include managing human surveillance and sample collection. We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Sincerely,

Dr Wan Hafizu Nazrin Bin Wan Mohamad Lotfi
Medical Officer of Pos Betau Health Clinic,
Kuala Lipis District Health Office,
27200 Kuala Lipis,
Pahang,
MALAYSIA

Email : (b) (6)

Fakulti Perubatan dan Sains Kesihatan
Faculty of Medicine and Health Sciences

UNIMAS/NC-21.26/03-01 (4)

18 June 2019

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

Dear Dr. Daszak,

This letter expresses the high interest that staff at Universiti Malaysia Sarawak has in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

The Universiti Malaysia Sarawak recognizes the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

We at Universiti Malaysia Sarawak have collaborated successfully with all partners on this proposal, and look forward to continuing our work together. Our work will include managing both human and animal surveillance, sample collection, testing, and development of laboratory diagnostics". We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Sincerely,

Dr Cheng Siang Tan (PhD, RBP)
Head, Centre for Tropical and Emerging Diseases
Faculty of Medicine and Health Sciences
Universiti Malaysia Sarawak
94300 Kota Samarahan
Sarawak, MALAYSIA
(b) (6)

UNIVERSITI MALAYSIA SARAWAK

06 June 2019

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

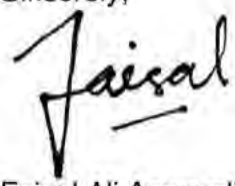
Dear Dr. Daszak,

This letter expresses the high interest of myself as a staff at Faculty of Resource Science and Technology Universiti Malaysia Sarawak, in a collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

I recognize the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

Colleague (Dr Tan Cheng Siang) and I from Universiti Malaysia Sarawak have collaborated successfully with all partners on this proposal and look forward to continuing our work together. Our work will include managing sample collection and testing. We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Sincerely,



Faisal Ali Anwarali Khan
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
94300 Kota Samarahan, Sarawak
MALAYSIA

(b) (6)

27 June 2019

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

Dear Dr. Daszak,

This letter expresses the high interest that staff at Klinik Kesihatan Bario has in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

The Klinik Kesihatan Baio recognizes the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

We at Klinik Kesihatan Bario have collaborated successfully with all partners on this proposal, and look forward to continuing our work together. Our work will include performing syndromic study by recruiting native patients with respiratory illness .We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Sincerely,

DR NADIA DIYANA BT HAMZAH
MEDICAL OFICER
KLINIK KESIHATAN BARIO
NO.10 PEKAN BARIO
98060 BARIO SARAWAK
(b) (6)

Laman Web Rasmi
HOSPITAL MIRI
Melayan anda... Layanan kekeluargaan

28 June 2019

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

Dear Dr. Daszak,

This letter expresses the high interest that staff at Hospital Miri, Sarawak has in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

The Hospital Miri, Sarawak recognizes the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

We at Hospital Miri, Sarawak have collaborated successfully with all partners on this proposal, and look forward to continuing our work together. Our work will include managing human surveillance, sample collection, testing, and development of laboratory diagnostics. We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Sincerely,

Dr. Ingrid Ting Pao Lin
Clinical Physician and researcher
Medical Department
Hospital Miri
Jalan Cahaya,
98000 Miri Sarawak
(b) (6)

UMS
UNIVERSITI MALAYSIA SABAH
BORNEO MEDICAL AND HEALTH RESEARCH CENTRE
FACULTY OF MEDICINE AND HEALTH SCIENCES
BLOCK E, LEVEL G
UNIVERSITI MALAYSIA SABAH
88400 KOTA KINABALU, SABAH, MALAYSIA
Tel :+6088-320 000 ext.611051
Faks :+6088-321 377/-321373
Email: [redacted]

Date : 15 **June** 2019

DR. PETER DASZAK
President
EcoHealth Alliance
460 W 34th St. 17th Floor
New York, NY 10001USA

Dear Dr. Daszak,

This letter expresses the high interest that staff at Borneo Medical and Health Research Centre (BMHRC) has in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

The BMHRC recognizes the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

We at BMHRC have collaborated successfully with all partners on this proposal and look forward to continuing our work together. Our work will include managing human surveillance, sample testing, and development & evaluation of laboratory diagnostics. We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Sincerely,

Kamruddin Ahmed

PROFESSOR DR. KAMRUDDIN AHMED
Director
Borneo Medical and Health Research Centre
Faculty of Medicine and Health Sciences
University Malaysia Sabah
Email: [redacted] (b) (6)

Cc. - File

Gleneagles™
KOTA KINABALU

Gleneagles Kota Kinabalu
A branch of Pantai Medical Centre Sdn Bhd (73056-D)
Riverson@Sembulan, Block A-1, Lorong Riverson@Sembulan,
88100 Kota Kinabalu, Sabah.
Tel : +6088 518 888 Fax : +6088 518 889
www.gleneagleskk.com.my

20 June 2019

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

Dear Dr. Daszak,

LETTER OF SUPPORT FOR THE PROPOSED NIAID EMERGING INFECTIOUS DISEASES RESEARCH CENTER (EIDRC) SUBMISSION (FOA: RFA-AI-19-028) ("Proposed Research")

This letter expresses the high interest that Gleneagles Kota Kinabalu Hospital, Sabah ("GKK") has in the collaborative research with EcoHealth Alliance and other partners on the Proposed Research titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

2. For your understanding, GKK recognizes the mutual benefits that could be gained through the research cooperation and successful partnership from the Proposed Research, including sharing of technology, samples, reagents, data and research results. The Proposed Research will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

3. Dr. Timothy William who is the Infectious Disease Consultant accredited by and practicing at GKK has collaborated successfully with all the partners on this Proposed Research, and look forward to continuing our collaboration. The collaboration will include managing patients, human surveillance and sample collection for diagnostic purposes. We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC. Should Dr. William and his colleagues succeed in being awarded the grant, he will work within the bounds of the regulations and ethics governing the conduct of the Proposed Research in Malaysia.

Kindly contact the undersigned should there be any inquiry. Thank you.

Yours sincerely,

Noel Cheah
Chief Executive Officer

'In the arms of one who cares'

UMS
UNIVERSITI MALAYSIA SABAH

HOSPITAL UNIVERSITI MALAYSIA SABAH
Fakulti Perubatan Dan Sains Kesihatan
Pejabat Pentadbiran HUMS, Aras 1, Blok A1,
Universiti Malaysia Sabah, Jalan UMS
88400 Kota Kinabalu, Sabah, Malaysia

Tel : (+6088-320000) samb 611705
Faks : (+6088-320377)
E-mel : hums@ums.edu.my

Our Ref.: UMS/HUMS6.11/100-1/4/249 ( )

Date : 20 June 2019

DR. PETER DASZAK
President
EcoHealth Alliance
460 W 34th St. 17th Floor
New York, NY 10001 USA

Dear Dr. Daszak,

This letter expresses the high interest that staff at Hospital Universiti Malaysia Sabah (HUMS) has in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

Hospital Universiti Malaysia Sabah, a 400 bedded hospital will be completed in June 2020. This hospital will run based on the concept of 'SMART Hospital', focusing on the delivery of high quality evidence based health care, using the latest technology and enhancing effective collaboration between health care providers. Patient centered care and collaboration will be at the centre of our processes. HUMS will also focus on health promotion and maintaining health, wellness and disease prevention

The Hospital UMS recognizes the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

We at HUMS are looking forward to collaborating successfully with all partners on this proposal. Our work will include managing human surveillance, sample collection, testing, and development of laboratory diagnostics. We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Yours faithfully,

PROFESSOR DR HELEN BENEDICT LASIMBANG
Chief Executive Officer

Telephone No. : (b) (6)
Fax No. : 088-321377
e-Mail Address : (b) (6)

HBL/clm

Pusat Penyelidikan Klinikal (CRC)
Hospital Queen Elizabeth
Karung Berkunci No. 2029
88586 Kota Kinabalu
Sabah, Malaysia.
088-517507 / samb. 7117, 7468 088-211906 crc.sabah@moh.gov.my

18 June 2019

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

Dear Dr. Daszak,

This letter expresses the high interest that staff at Queen Elizabeth Hospital has in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

The Queen Elizabeth Hospital recognizes the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

We at Queen Elizabeth Hospital have collaborated successfully with all partners on this proposal and look forward to continuing our work together. Our work will include managing human surveillance and sample collection. We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Sincerely,

DR. NAGARAJAN NAGALINGAM
MMC 40081
KETUA UNIT RAWATAN KESAKITAN &
KETUA UNIT CRC
HOSPITAL QUEEN ELIZABETH 1
KOTA KINABALU, SABAH.

13/6/19

Dr. Nagarajan A/L Nagalingam
Head of Unit,
Clinical Research Center (CRC),
Queen Elizabeth Hospital,
Karung Berkunci No. 2029,
88586 Kota Kinabalu,
Sabah, Malaysia
Email: (b) (6)

CRC
SABAH, MINISTRY OF HEALTH MALAYSIA
Research that matters to patients

Pusat Penyelidikan Klinikal (CRC)
Hospital Queen Elizabeth
Karung Berkunci No. 2029
88586 Kota Kinabalu
Sabah, Malaysia.
☎ 088-517507 / samb. 7117, 7468 🖷 088-211906 ✉ crc.sabah@moh.gov.my

06 June 2019

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

Dear Dr. Daszak,

This letter expresses the high interest of myself a staff at Sabah State Health Department has in collaborative research with EcoHealth Alliance and other partners on the proposed NIAID Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

I recognize the mutual benefits to be gained through research cooperation and a successful partnership in this center, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

I and other colleagues from Sabah State Health Department have collaborated successfully with all partners on this proposal, and look forward to continuing our work together. Our work will include managing human surveillance, sample collection, testing, and development of laboratory diagnostics. We will also actively take part in technology transfer and research cross-training with other partners, and are ready, willing and able to work in surge capacity to conduct research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Sincerely,

Dr Giri Shan Rajahram
Infectious Disease Physician
Queen Elizabeth Hospital
Sabah State Health Department
Locked Bag 2029, 88586,
Kota Kinabalu, Sabah,
Malaysia
(b) (6)

LEE KONG CHIAN SCHOOL OF MEDICINE

NANYANG TECHNOLOGICAL UNIVERSITY SINGAPORE

Imperial College London

REDEFINING MEDICINE, TRANSFORMING HEALTHCARE

14th June 2019

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

Dear Dr. Daszak,

Letter of Support for the Application of NIH NIAID Emerging Infectious Diseases Research Center (EIDRC) – 'Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia'

I am pleased to offer the support of the Lee Kong Chian School of Medicine (LKCMedicine), Nanyang Technological University to National Institutes of Health, National Institute of Allergy and Infectious Diseases (NIH NIAID) Emerging Infectious Diseases Research Center (EIDRC) submission (FOA: RFA-AI-19-028), titled 'Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia'.

LKCMedicine understands that it will participate officially as a collaborating institution and that A/Prof Yeo Tsin Wen will be listed as a Co-Investigator of the grant. A/Prof Yeo's involvement in the project will be to work with local stakeholders and collaborators to supervise and co-ordinate the clinical field studies in Malaysian Borneo, as well as offering sequencing facilities to detect novel pathogens.

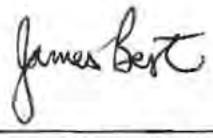
LKCMedicine recognises the mutual benefits to be gained through research cooperation and a successful partnership in this proposed entreprise, including sharing of technology, samples, reagents, data and research results. The research proposed will advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause illnesses that may have been previously unreported, or undiagnosed. We believe the results will be scientifically important, and have great public health value in the region, and globally, by identifying key pandemic threats in an EID hotspot region.

A/Prof Yeo at LKCMedicine has collaborated successfully with partners on previous projects and proposals, and is looking forward to continuing the work together. The work will include clinical studies to establish the risk of crossover zoonotic infections, and development of novel serological and molecular tools to test for viral spillover. A/Prof Yeo will also actively take part in technology transfer and research cross-training with other partners.

LKCMedicine will support A/Prof Yeo's proposed field work in Malaysian Borneo and also has a range of facilities to support the conduct of further experiments to detect novel pathogens, which includes the laboratory space, multiple sequencers and bioinformatic support as well as other research facilities to conduct the project.

Overall, A/Prof Yeo is an independent principal investigator at LKCMedicine and runs a functional laboratory at the School. The School and I fully support his application for the grant.

Yours sincerely,



Professor James D. Best
Dean, Lee Kong Chian School of Medicine
Nanyang Technological University

U.S. CENTERS FOR DISEASE CONTROL AND PREVENTION
SOUTHEAST ASIA REGIONAL OFFICE

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

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

Ref: RFA-AI-19-028
Title: Understanding Risk of Zoonotic Virus Emergence in EID Hotspots of Southeast Asia

Dear Dr. Daszak:

On behalf of the U.S. Centers for Disease Control and Prevention Southeast Asia Regional Office (CDC-SARO) we would like to offer our enthusiastic support for your application to the National Institutes of Health (NIH) proposing a systematic approach to better understand the diversity and epidemiology emerging viruses from the Coronavirus, Paramyxyovirus, and Filovirus families and commitment and improve laboratory capacity to identify and diagnose these pathogens.

CDC has a long history of collaboration with government organizations, non-governmental organizations, academic institutions including success with the NIH-funded research activities. I believe that CDC's work in Southeast Asia, through CDC-SARO, provides an effective platform to successfully collaborate with you on the proposed "Understanding Risk of Zoonotic Virus Emergence in EID Hotspots of Southeast Asia" project.

I understand that the overall goal of this proposed study is to further understand an important group of emerging zoonotic viruses that threaten global health security. The mission of CDC's global health presence is to protect Americans and people worldwide from public health threats by working with partners to build capacity, advance research, and respond in times of crises. This project will advance CDC's global mission by improving our knowledge of the zoonotic epidemiology of potentially highly-pathogenic emerging viruses, understanding the characteristics of these viruses that may be associated with increased potential for spill over and/or pathogenesis in humans, as well as improving our ability to detect and diagnose these unique pathogens. The emergence of Nipah virus in Malaysia, SARS-CoV, zoonotic influenza viruses (H5N1, H7N9) MERS-CoV, Zika, and finally Ebola in West Africa demonstrates the zoonotic potential of these and other viruses and highlights the critical gaps in knowledge including well-characterized diagnostics and medical countermeasures to stop these public health threats.. Furthermore, the spread of these outbreaks across international borders provides clear evidence of mobility of these pathogens given current trade/movement of animals and extensive networks of international travel.

Should you be successful with your application, CDC SARO is willing to provide technical support, liaise regional Ministries and CDC country offices, and provide general assistance as needed to advance the proposed science.

3rd-5th floor, Building 7, Department of Disease Control, Ministry of Public Health, Tivanon Road, Nonthaburi, Thailand 11000

Telephone : (66-2) 580-0668, 580-0669, Fax: (66-2) 591-5443

I wish you all the best for the application and look forward to working together to reduce the threat of emerging viruses in Southeast Asia.

Sincerely yours,

John MacArthur, MD, MPH
Director, Southeast Asia Regional Office
Centers for Disease Control and Prevention
Representative to Thailand, Department of Health and Human Services

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

05 June 2019

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

Dear Peter,

I strongly support the collaborative research proposed by EcoHealth Alliance and other partners on the NIAID EIDRC proposal titled "Understanding risk of zoonotic virus emergence in EID hotspots of Southeast Asia".

As you know, I have long experience with both government and university sectors in researching wildlife-associated EIDs including henipaviruses, coronaviruses and filoviruses in Southeast Asia and China. As an Honorary Professor in the School of Veterinary Science at UQ, the mutual benefits to be gained through research cooperation and partnership in this project, including sharing of technology, samples, reagents, data and research results, are clearly evident. The proposed research will undoubtedly advance our understanding of the ability of wildlife-origin coronaviruses, henipaviruses and filoviruses to spillover into high-risk human populations and cause morbidity and mortality previously unreported or undiagnosed. The results will be scientifically important, and have direct and significant public health value both regionally and globally, by identifying key pandemic threats in an EID hotspot region.

I very much look forward to participating in this new initiative with previous partners with a proven track record in this field. My contribution will include expert input in relevant study design, data collection and analysis, partner meetings, and dissemination and publication, and more broadly, technology transfer and research cross-training with other partners, as well as contribute surge capacity to undertake research during outbreak situations, in collaboration with all other EIDRCs and the EIDCC.

Yours sincerely,

Dr. Hume Field BVSc MSc PhD MACVS

Honorary Professor
The University of Queensland | School of Veterinary Science
Brisbane, Australia
(b) (6)

RESOURCE SHARING PLAN

To share resources with the academic research community, we will use the uniform Material Transfer Agreement (MTA), which acknowledges that the materials are proprietary to Institutions of the Cooperative Agreement and permitting their use in a manner that is consistent with the Bayh-Dole Act and NIH funding requirements. NIH research grants require that research be made available to the scientific community and public. The primary method of data sharing is through peer-reviewed publications in scientific journals and by presentation at scientific meetings. In addition, data and results created from NIH supported research will be submitted to NIH in annual progress reports per the terms and conditions of this award. Recombinant viruses, transgenic mouse models and experimental recombinant protein constructs will be made available upon request following a standard procedure (below). Several viruses isolated and studied in this program are select agents so these viruses will not be shipped unless appropriate documentation demonstrates the existence of approved BSL3/4 facilities, select agent licenses, and shipment using approved CDC and Department of Commerce procedures.

We already have established MTAs between most of our EID-SEARCH, consortium partners and will ensure these agreements are up to date and agreed upon by our consortium at the start of our project and then reviewed annually. Having these agreements in place will further reduce the time needed to share reagents and other resources in the event of an outbreak when time-sensitive sharing of biological resources and diagnostic reagents is most critical. **At the start of the project, we will work with the EIDRC – Coordinating Center to ensure these agreements and resource sharing plans are compliant and aligned with plans created for NIH's other EIDRCs.**

Data Sharing Plan

EcoHealth Alliance (EHA) will house the Data Management and Analysis (DMA) team for EIDRC SEARCH, led by Co-PIs Olival and Zambrana-Toreillo and include Key Personnel Latinne and Mendelsohn. EHA has served as the data and analysis hub for numerous multi-institutional, multi-sectoral, international disease research groups, including acting as the Modeling and Analytics lead institution for the USAID-PREDICT project, the Western Asia Bat Research Network lead by co-I Olival (*1*) and EHA's Rift Valley Fever Consortium (*2*). We will leverage our experience and infrastructure from those projects.

Project Database: We will create a dedicated, centralized EIDRC database to ingest, store, link, and provide for analysis all data associated with the proposed study and other expanded projects associated with the research network. The database will be SQL-based and use encrypted, secure cloud hosting services and enable export to archival and platform-independent formats. It will ensure data and metadata compatibility between project components, track data versioning and annotations. The system will be designed to work with both with both paper- and tablet-based field data entry and with laboratory information management systems in place in individual partner labs. We will design and engineer the systems to be compatible with other NIAID approved data management systems, including those utilized by the EIDRC-CC, by designing secure APIs, and matching data fields and data standards. The database will use existing metadata standards, including NCBI standards for genetic and molecular data and Ecological Metadata Language (EML) for field and wildlife data, as well as other standards and formats designated by the EIRDC CC. This will enable rapid publication and deposition of data. Granular security and privacy controls will be applied so that specific expansion projects undertaken in the network may be managed while maintaining data confidentiality as needed.

Data Identification and Privacy: For human clinical data and questionnaires, data will be identified by a unique identification code assigned to each individual and only this, de-identified code will be accessible in the project database. All questionnaire data and biological samples will be labeled with a unique alphanumeric identification code, assigned to each enrolled, sampled individual that does not identify the individual from whom data are collected. Participants' names and codes, along with other records with identifying information such as informed consent forms, will be stored in a separately secure system accessible to only essential project staff. If participants agree during the consent process, they may be contacted about having their samples or questionnaire data used for future separate studies about new animal infections discovered in the

future, and factors that may affect their chances of getting these animal infections. No data will be released for other purposes without full consent from participants. Upon completion of the project, personal identifying information will be destroyed unless this protocol is extended.

Training: Members of the DMA team will team will develop documentation and provide training for field and laboratory teams at all partner institutions in data management, metadata standards and data hygiene best practices. The DMA team will act as trainers and consultants for partner institutions in experimental design, power analysis, data analysis, and computational and reproducibility issues. DMA trainers will visit each partner institution and/or field team base for training workshops and analysis consultations, and partner institution researchers and students will spend extended time at EHA for collaborative analysis, a model that has been successful in building and maintaining analytical capacity under our NSF EcoHealthNet and PREDICT programs.

Computing Resources: EHA operates a cluster of high-performance servers (System76 20- and 36-core Linux servers with NVIDIA deep-learning GPUs), for data analysis activities, as well as infrastructure to launch cloud-based computing environments of virtual machine with identical software infrastructure. Our servers provide a web-based analysis environment with all necessary software for statistical and bioninformatics work that is available to the DMA and partners anywhere in the world. We use a mixture of cloud services (AWS, Azure, Backblaze, GitHub) to provide redundancy, backup, version control, and rapid post-disaster recovery. The cluster is available to all project partners and can be used use for both high-performance and training-level work (under isolated environments for security and performance).

Data and Code Sharing: 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. Additional ecological data collected in wildlife sampling will be deposited to 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.

Sharing Model Organisms

Within the program, we will utilize standard laboratory mice as well as different Collaborative Cross mouse strains as well as various transgenic mouse strains, several of which are already available at the NIH-supported Mutant Mouse Regional Resource Center (MMMRC) at UNC. The Collaborative Cross mice are already publicly available from the UNC Systems Genetics Core Facility and the Jackson laboratories, and as such available to the scientific community. All genotyping information generated on these populations will be deposited in the appropriate public repositories (e.g. GEO, ImmPort, ENA). Similarly, all phenotypic data generated within this program from studies with mice will be deposited in the Mouse Phenome Database upon publication, as well as ImmPort to ensure dissemination to the community at large.

In accordance with the NIH/NIAID data sharing and release guidelines, we will coordinate the rapid and unrestricted sharing of all data generated as part of this project.

1. Genotypes generated on the MUGA mouse array, including raw x- and y- intensity data and derived genotype calls will be made available for download from the Mutant Mouse Regional Resource Center at UNC's website (https://www.med.unc.edu/mmrrc/genotypes/publications) and at Zenodo (https://zenodo.org/).

Reagent Sharing

For all other reagents/requests, we have established a consistent process for evaluating requests for samples and reagents from outside scientists. In order of priority, these include: 1) requests for reagents that have been

published in peer-reviewed journals; 2) requests which enhance/promote a specific agenda of the program projects and faculty; 3) requests that promote scientifically valid collaborations between project faculty and outside scientists; and 4) overall research and public health needs. The general format involves: a) establishing a working knowledge of the research agenda and credentials of the requestor, b) group discussion and agreement, 3) MTA agreement with the appropriate institution, or license agreement with a commercial entity, and 4) inventory checking and sending out of reagents. We will work closely with the appropriate institutional Technology Transfer Office and individuals involved in these transactions. The goal will be to provide reagents within a few months of receiving a request for traditional research purposes. In the event of an outbreak or emergency situation, we will communicate with the NIH and EIDRC-CC, and rapidly speed up resource sharing among our EID-SEARCH core partners and our extended network. As documented in the Research Strategy, EHA has successfully provided rapid technical assistance for testing and reagent needs during outbreaks under the USAID-PREDICT project, and has strong existing relationships and existing MTAs with our core EID-SEARCH partners to facilitate this. If needed, we will also acquire appropriate letters from the recipient institutions environmental health and safety officers and help coordinate CDC and/or USDA and Department of Commerce permits. The program faculty will not send reagents to individuals or institutions that do not have appropriate documentation of appropriate containment for the materials, might harbor ill-intentions, or are conducting irresponsible research.

Genomic Data Sharing

We will ensure compliance with NIH's Genomic Data Sharing plans for all viral sequence data generated in this project. We anticipate obtaining genetic sequence data for 100s of novel virus genotypes, including RNA-dependent RNA polymerase (RdRp) sequences for all strains/genotypes and sequences of viral attachment glycoproteins. We will generate full viral genomes for a subset of the viruses and human virus strains that we identify. We will also generate host genetic sequence data for relevant cellular receptor genes of wildlife species. We will deposit all genetic sequences in the NIH data bank, NCBI GenBank as soon as possible after data are generated (including ensurance of quality control), and no later than 6 months, so that they are readily available to the scientific community. 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. We anticipate sequence generation will occur over the 5 year proposed project period.

All datasets and associated meta-data will be additionally submitted to Virus Pathogen Resource (ViPR, http://www.viprbrc.org). All computational models of biological processes will be made available on the BioModels Database (http://www.ebi.ac.uk/biomodels-main/).

Intellectual Property

Intellectual property agreements, identified during the course of this project, will be accomplished by negotiation in good faith among the institutions and inventors. We will work with the inventors in the production of the necessary documents, working with the particular institutions, legal firms and commercial interests. It is anticipated that companies and institutions will have access to these reagents and viruses by MTA (for research purposes) or by a license agreement to be negotiated in good faith with a company.

Literature Cited

1. K. Phelps *et al.*, Bat Research Networks and Viral Surveillance: Gaps and Opportunities in Western Asia. *Viruses* **11**, (2019).
2. V. Msimang *et al.*, Rift Valley Fever Virus Exposure amongst Farmers, Farm Workers, and Veterinary Professionals in Central South Africa. *Viruses* **11**, (2019).

AUTHENTICATION OF KEY BIOLOGICAL RESOURCES AND SCIENTIFIC RIGOR

EcoHealth Alliance will actively engage with each partner laboratory to ensure that the highest quality of science, public accountability, and social responsibility in the conduct of science is maintained throughout this project. The overall goal is to ensure that the underlying scientific foundation of research conducted under our EID-SEARCH project from conception to completion is scientifically sound. The application is designed to ensure rigor, by using a robust and unbiased experimental design, well defined methodology, large group sizes that ensures statistical rigor and analysis, clearly interpretable endpoints, biological authentication of key biological resources and transparency in the reporting of results. 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 statistical justification of study design throughout the duration of the award. We have highlighted our statistical approach to analysis of animal and human data in our Research Strategy and accompanying Statistical Analysis Plan. Whenever possible, multiple experimental approaches are used to demonstrate congruency in data interpretation. We will ensure that experimental designs will include considerations of sex as a "Relevant Biological Variable" in all studies involving human subjects or vertebrate animals. Unless otherwise specified and justified, all sampling and screening will include male and females.

Details below ensure the authentication of key biological resources needed for further biological characterization of viruses in the laboratories of the University of North Carolina, Duke-NUS Medical School, Singapore, and National Emerging Infectious Diseases Laboratories (NEIDL), Boston (Aim 1) as well as authentication of key reagents needed for molecular and serological screening, particularly the multiplex microsphere immunoassay (MMIA) or Luminex assay reagents developed by Uniformed Services University, Bethesda (Aims 2 and 3).

Cell lines. We will purchase cell lines from commercial vendors (e.g., ATCC), which confirm the authentication of the cells they supply using short tandem repeat (STR) profiling (ATCC). For all cell lines, we will create a low-passage (<5 passages) working stock for use across all experiments, and while in use, we will monitor morphology and growth kinetics continuously and perform mycoplasma tests monthly. If cultures exhibit unexpected changes in growth or morphology or test positive for mycoplasma, we will discard them immediately. All genetically modified cell lines will be frozen at low passage and maintained in culture only for 10 passage cycles. Once thawed and placed in culture, each cell line will be re-evaluated for maintenance of gene targeting. Primary cells. Early passage primary lung cells from humans are a key reagent for the proposed studies, including airway epithelial cells and microvascular endothelial cells. Human cells are derived from donors of both sexes and from all ages and ethnic groups and are provided from deidentified donors from the cystic fibrosis center tissue procurement fee for service center (https://www.med.unc.edu/marsicolunginstitute/core-facilities/tissueprocurmentandcellculturecore/). 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).

The FreeStyle™ 293-F cell line is used for the development and expression of the viral recombinant proteins. These cells are from Thermo-Fisher Scientific, Inc., and are adapted to suspension culture in FreeStyle™ 293 Expression Medium.

Luminex (MMIA) Serological Reagents. To improve CoV-specific antibody detection, we propose to develop a multiplex microsphere immunoassay (MMIA) to enable serum samples to simultaneously be tested for antibodies reactive to the trimeric S, monomeric S_1 and S_2 subunits, and N. This assay development, being led by Uniformed Services University (USU), will involve some non-standard biological and chemical resources that

require validation and authentication. USU has specialized strategies to oversee the authentication of key biological resources, reagents and chemical resources during assay development and validation. Each purified protein antigen will be tested by protein gel and Western blot analysis to confirm expected protein size and weight. Polyclonal rabbit antisera specific to each purified antigen will be generated and used to test size exclusion and affinity purified proteins with Luminex-based platform. These polyclonal rabbit antisera will act as positive and negative controls for each purified protein and will be used to quantify inter and intra assay variation as well as ensure that each batch of purified protein retains the same level of assay reactivity. This positive and negative control assay standardization will be transferred to both international partner laboratories (JUST, Lugar) and guidelines detailing monthly testing will be included as part of the in-country training. Assay control results between USU and implementing laboratories in Thailand and Malaysia will be compared throughout the project to identify and control of any user or assay errors at each site.

Plasmids. We will sequence all cloned genes after their generation, after each PCR amplification, and after other modifications (such as site-directed mutagenesis). All molecular clones will be synthesized using commercial vendors and sequence verified after receipt. All mutations will be verified prior to assembly of full length clones for recombinant virus recover, which includes SARS-CoV, MERS-CoV, SARS-like and MERS-like CoV, select bat coronaviruses, Ebola and Ebola related filoviruses, Marburg and Marburg related filoviruses, and henipaviruses (Nipah, Hendra and related viruses).

Viruses. We will sequence wildtype and recombinant virus stocks to confirm the absence of unwanted mutations. For experiments in mice, each stock of live virus is deep-sequenced to confirm its authenticity, tested for the absence of mycoplasma, and tested for its lethality in animal models.

Animal Models. We will breed wild-type (WT) C57BL/6J, Collaborative Cross (CC) Recombinant Inbred Mice, and various transgenic C57BL6/J mice expressing the hACE2 receptor and humanized C57Bl6/J 288/330$^{+/+}$ DPP4 mice, which can be used to evaluate pathogenic outcomes following SARS-CoV, bat SARSr-CoV and MERS-CoV and bat MERSr-CoV. Animals will be used at UNC and Duke-NUS or will be shipped to the NEIDL in Boston Biosafety Level 4 (BSL-4) facility for experiments with authentic Ebola, related filoviruses and various henipaviruses. We obtained founder mice for the WT C57BL/6J colony from Jackson Laboratories, which confirms the authenticity of the animals they supply, while all CC mice were obtained from the University of North Carolina CC genetic reference population (https://csbio.unc.edu/CCstatus/index.py).

For assay development at USU, polyclonal rabbit sera specific to the recombinant viral glycoprotein to be generated will be prepared by Noble Life Sciences Inc., Maryland; a fee-for-service and AAALAC accredited, OLAW assured, and USDA licensed company with over 50 years of experience in animal housing and husbandry for large and small animals.

Mouse strain Genetic Validation. Inbred mouse strains and Collaborative Cross Mice are an invaluable tool for biomedical research, and represent a key aspect of this entire program. 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 the cost effectiveness of 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.

Mice with novel and interesting phenotypes after infection with different program viruses of interest will be deposited at the MMRRC housed at the University of North Carolina. The MMRRC is the nation's premier national public repository system for mutant mice. Funded by the NIH continuously since 1999, the MMRRC

archives and distributes scientifically valuable spontaneous and induced mutant mouse strains and ES cell lines for use by the biomedical research community. The University of North Carolina is one of the 4 major academic MMRRC centers across the nation.

Genome Sequencing. All PCR assays will be performed using appropriate control materials. Synthetic DNA constructs will be designed and used as universal controls. These DNA plasmids will contain short regions of overlapping viral sequences that act as primer binding sites for different PCR assays. The sequence spanning any two primers binding regions will be unique, allowing for contamination to be readily identified. Contamination control PCR will be used to rule out the possibility of contamination in a sample with universal control when a positive PCR result is obtained. All DNA sequence reads obtained from PCR screening of wildlife and human specimens will be examined by bioinformatic leads in each partner laboratory upon generation and again upon integration into our database system, for completeness, accuracy, and logical consistency. When base calls are uncertain, chromatograms will be reviewed to resolve ambiguities; and cloning and re-sequencing of samples if disagreement is observed. Once all test results (e.g., initial detection by PCR and subsequent sequencing of viruses) are available for a given specimen, the results are interpreted in light of all available and up-to-date scientific literature and previous findings by experienced EID-SEARCH virologists and bioinformaticians. This iterative process ensures the highest quality, most robust, data possible. We will ensure completeness of all existing metadata, meeting NCBI standards for genetic and molecular data.
For authentication of whole genome sequencing using next generation sequencing methods, we will ensure standard QC metrics are met at all points in the process of data generation, including: 1) sample quality control - validation of nucleic acid quality using spectrophotometric methods; 2) QC on base read quality and sequence reads through analysis of quality scores (Q scores); 3) standard methods to ensure quality of assembled/aligned reads by using NCBI and ICTV recognized viral reference sequences.

Following details in our Genomic Data Sharing plans, we will deposit all genetic sequences in the NIH data bank, NCBI GenBank as soon as possible after quality control process is completed, and no later than 6 months after data have been generated, so that they are readily available to the scientific community. All datasets and associated meta-data will be additionally submitted to Virus Pathogen Resource (ViPR, http://www.viprbrc.org) where they can be further authenticated by the scientific research community.

Intellectual Property. Intellectual property agreements, identified during the course of this project, will be accomplished by negotiation in good faith among the institutions and inventors.