Dear Pat and Eun Chung,

It was wonderful to see you in Ft. Collins. I'm grateful that we had time to talk about this project and for your interest and support. Attached are two briefs which detail the scope of work and scientific rationale for setting up the Pteropus colony. Let's use this as a starting point for further discussion about a potential contract. I'd be happy to provide additional information as per your guidance.

Cheers,
Jon

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Jonathan H. Epstein DVM, MPH, PhD
Vice President for Science and Outreach
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

web: ecohealthalliance.org

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.
Establishment of a pteropid bat colony (*Pteropus medius*) in the United States to study host-virus interactions, including the immune response, to Nipah virus and other zoonotic pathogens that threaten human health.

Prepared by
Jonathan Epstein, DVM, MPH, PhD, EcoHealth Alliance
Tony Schountz, PhD, Colorado State University
Dr. Vincent Munster, PhD, NIH NIAID Rocky Mountain Laboratories

Bats have been shown to carry more zoonotic pathogens than any other mammalian taxon (Olival et al, Nature 2017).

Several emerging zoonotic pathogens associated with severe human disease originated, are hosted or suspected to be hosted by bats, including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS CoV), Marburg virus, ebolaviruses, and a wide range of lyssaviruses. We recently found evidence of a SARS-like coronavirus in Chinese horseshoe bats that has the capability to transmit directly to people, which suggests that the original transmission of SARS may have been directly from bats, rather than via civets or other animal intermediate hosts. Recent studies have also found evidence that bats were reservoirs the ancestors of other human pathogens such as hantaviruses, hepatitis C, rubeola, mumps and rubella viruses. Much of this work arose from phylogenetic, epidemiological and virological studies of viruses identified in wild caught bats, including substantial work from our group. These findings have generated larger questions about how bats (the second largest group of mammals with more than 1,200 species) can host these viruses that without substantial pathology, yet they cause substantial disease in other species, including humans.

To determine whether bats have a specialized physiology or immune systems that permit viral infection with minimal disease requires development of bat models that can be used in laboratory experiments.

Bats of the genus *Pteropus* (family Pteropodidae) comprise more than 60 species that range from Madagascar eastward through most of Asia, Australia, and the Pacific islands. Several species of pteropid bats are natural reservoirs of NiV and other henipaviruses, including Hendra virus (HeV) and Cedar virus in Australia. Both Nipah and Hendra viruses are biosafety level 4 pathogens and select agents. Currently, the only captive colony of pteropid bats available for infectious disease research (to our knowledge) exists at the Australian Animal Health Laboratory (AAHL) in Geelong, Australia, which has BSL-4 small and large animal facilities. Although AAHL has developed and will collaboratively share cell lines derived from one species of pteropid bat (*P. alecto*), at present the bats are not available to researchers outside of AAHL. Thus, a significant need remains for a lab animal model that can be used to study NiV and HeV host-virus interactions and generate additional laboratory reagents and resources available to a broader research community.

*Pteropus medius*, in particular, is of special interest for viral research because it is has been found to carry Nipah virus and other viruses with potential human health impact, including filoviruses and other uncharacterized henipaviruses for which we have serological evidence. This species also carries a recently discovered virus called GBV-D, a flavivirus related to Hepatitis C virus. *The propensity for this particular species to carry a wide spectrum of viruses*
related to known human pathogens (without clinical affect) makes it an ideal candidate as a laboratory model to advance immunological and virological studies in bats.

The establishment of a research colony of Indian flying foxes (Pteropus medius) is critical to facilitate research in the United States that will test hypotheses related to the cellular mechanics of Nipah virus (NiV) and the host immune response, in vivo, in a wildlife reservoir species for Nipah virus. The Indian flying fox is endemic to the Indian subcontinent, and widely distributed throughout Bangladesh and India, where more than fifteen outbreaks of Nipah virus encephalitis have been reported since 2001. There are no bats available in the United States for research related to Pteropus physiology, immunology, and viral pathophysiology. NiV is an emerging, high consequence pathogen with 75% - 100% mortality in humans in Bangladesh, where is causes seasonal outbreaks of encephalitis. Currently, there is no effective treatment or vaccine for NiV. It is a highly communicable disease, including person-to-person and nosocomial transmission. Though the majority of outbreaks, to date, have occurred in rural villages, Bangladeshi patients are often transported to Dhaka for care. The introduction of NiV to Dhaka, a city of 12 million people with an international airport linking major cities, including New York, London, and Hong Kong, represents one of the most significant factors contributing to Nipah virus' pandemic potential.

Maintaining bat colonies requires many specialized husbandry facilities and resources. Indeed, insectivorous bats are notoriously difficult to keep, let alone breed in captivity. Frugivorous bats are much easier to maintain in captivity. They are typically robust and will eat a variety of fruits that are readily available in the United States. Their social structure and behavior is well understood, and zoological institutions have successfully kept and bred a variety of fruit bats species, including many different pteropid bat species. [Note: in the context of this proposal, zoological institutions are not a viable source of bats for founding a colony as biomedical research is generally considered “off mission” for zoological gardens focused on species conservation] The Indian flying fox is an attractive bat model because it is a reservoir host of NiV, its large body mass (~700-900g) allows for relatively large volumes of blood and lymphoid cells to be safely sampled to support clinical research, its conservation status is “non-threatened” (thus allowing wild founders to be more readily sourced), and it is easy to maintain and breed in captivity.

2) Who will establish the colony? Where would the bats come from and where would the colony be maintained?

Our group includes experts on the behavior and husbandry of bats, their ecology, the epidemiology of Nipah virus in wild populations, and the design and implementation of experiments involving non-traditional animal models.

Colorado State University is a registered NIAID contractor for establishing lab animal models and will be the location of the proposed bat colony. Tony Schountz, PhD is an Associate Professor in the Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine at CSU. Dr. Schountz previously established a breeding colony of, Jamaican fruit bats (Artibeus jamicensis) that has been used for Tacaribe virus and MERS-CoV experimental research. CSU currently has the facilities to establish a colony of Pteropus medius and Dr. Schountz and Richard Bowen, DVM, PhD will be responsible for establishing and maintaining the research colony. The Director of Laboratory Animal Services at CSU is Lon Kendall, DVM, PhD, who has overseen the veterinary care of the Jamaican fruit bat colony. Thus, the facilities and staffing expertise are already in place at CSU for working with bat colonies.
Dr. Jonathan Epstein, a veterinary epidemiologist at EcoHealth Alliance, has nearly 20 years of experience working with pteropid fruit bats in the wild. His research has focused on the epidemiology and ecology of Nipah virus and other zoonotic agents in bats. He directed the capture, quarantine and transport of live *Pteropus vampyrus* from Malaysia (another reservoir of Nipah virus) to AAHL as part of an NIH-funded long-term study of henipaviruses in bats in 2005. He has been working in Bangladesh since 2006, and has established strong collaboration with the government of Bangladesh, including the federal wildlife authority. Dr. Epstein, and his team in Bangladesh will be responsible for the capture, quarantine, and transportation of the bats from Bangladesh to CSU (Fort Collins, Colorado). He will also provide guidance for the facility at CSU (e.g., diurnal cycles, feeding, enrichment, etc.). Dr. Epstein is currently collaborating with Drs. Schountz and Munster on bat immunology studies and will continue to provide leadership and scientific engagement in this and future collaborative studies related to bat immunology and virology related to the imported *Pteropus* bats.

Dr. Vincent Munster is a senior scientist in the Laboratory of Virology, Rocky Mountain Laboratories, NIAID, (Hamilton, MT). His work has focused on experimental studies of bat-borne high containment pathogens such as Ebola virus, Nipah virus, SARS-CoV and MERS-CoV. Dr. Munster will facilitate the establishment of the colony, and will be the laboratory lead and co-investigator on all experimental studies utilizing these bats. We will have the support and use of the BSL 4 laboratory and veterinary personnel at RML for experimental work utilizing the bats.

Mr. Brian Pope, the Director of the Lubee Bat Conservancy in Gainesville Florida has more than 12 years of bat husbandry experience at zoological parks, including Disney World's Animal Kingdom, and will provide expert guidance on the regulatory aspects of bat importation and the development of the internal environment for the bat colony. He and his staff will provide training to the veterinary and animal care staff at CSU and RML on the husbandry and care of the bats. Mr. Pope and Dr. Epstein have collaborated for more than five years on bat immunology studies at the Lubee Bat Conservancy, and Dr. Epstein currently serves on Lubee's Scientific Advisory Board.

To found the colony, we propose to import 40 adult *P. medius* from Bangladesh, with the support of the Forestry Department – the federal wildlife agency. We will import 36 pregnant female bats, and 4 males - all seronegative for Nipah virus. A temporary quarantine facility will be constructed by the Forest Department at the Dhaka zoo, where veterinary and animal care staff are available. Bats will be sampled (blood and urine) every three weeks and samples will be sent to RML laboratories and tested for Nipah virus antibodies and RNA using ELISA, SNT, and PCR. Bats that have three consecutive negative tests will be shipped to CSU. Our group previously transported pteropid bats from Malaysia to Australia for research purposes. *P. medius* is a seasonal breeder, and females within a colony tend to be pregnant all at once, so capturing 35 pregnant females is achievable. The gestation period is six months, and the timing of transport will be such that the bats will be in the fourth month of pregnancy to maximize the safety to the fetus during transport. We expect 80-90% of pregnancies to be maintained during quarantine and shipment, such that the colony will immediately provide about 30 juveniles that could be used for experimental work within 12 months of birth or to continue breeding after 30 months when they reach sexual maturity. The adults will be bred every year (one breeding cycle per year), which will generate a cohort of 20-35 bats each season. Over a period of 3-5 years, we expect to have generated a colony of more than 200 bats that will be available for experimental studies.
3) Long-term sustainability.

Use of the bat colony as well as cell lines derived from bat tissues will be made available to the scientific community. We expect that cell lines will be the most frequently requested products that could be readily shared among the scientific community. Have a supply of primary and immortalized (e.g., large T, hTERT) cell lines in the US will rapidly facilitate research because it will obviate the need for CITES and other import permits when reagents are shipped to other US-based labs. The colony will also benefit conservation efforts by providing genetic material to zoological institutions that have breeding programs for *P. medius* now or in the future.

Support from NIAID will be required to establish and expand the colony over an initial 5-year period. Once the colony is established, we will generate reagents and cell lines that will be made available to other researchers upon request. After the completion of the contract and to support the maintenance of the colony and associated resources, we will establish a modest fee structure for use of the bats and materials derived from the bats, which will be channeled directly back into colony maintenance costs. We will also consider experiments that require the use of bats and will include budget in each proposal that will be used to support maintenance costs. The fee structure could be modeled from the one used by the Lubee Bat Conservancy, or a de novo fee-for-service system will be developed.