

regular conference calls and in-person meetings to facilitate rapid decision making within the EID-SEARCH. **This committee will also convene to manage EID-SEARCH response to outbreaks.**

**4.1.b Project Management in Thailand and Malaysia:** Wacharapluesedee and Hughes have collaborated directly with EHA for >15 years, including acting as country coordinators on the USAID PREDICT project for the last 10 years (project end date Sept. 2019). They maintain strong ties with Ministries of Health (MOH), Agriculture and Environment, multiple universities and research institutions, clinics, and hospitals, in their respective countries and across the region. The EID-SEARCH will use these connections to disseminate results, obtain permissions to conduct sampling, and also rapidly respond to and assist with outbreaks as they happen. Peninsular Malaysia, Sarawak, and Sabah are the three main Malaysian administrative regions, and effectively operate as three separate countries, with different regulations and government structures. We therefore provide specific details on the management of EID-SEARCH activities in each:

Coordination among Peninsular Malaysia, Sabah and Sarawak will be led by co-I Hughes (Conservation Medicine Ltd), and follow a successful model we implemented under USAID-PREDICT. **On Peninsular Malaysia** this project will be administered through the Zoonosis Technical Working Committee (ZTWC) established under the PREDICT project with a binding MOU among EHA, CM Ltd. and ZTWC, and including officers from MOH, Dept. of Veterinary Services, and PERHILITAN (the Govt. wildlife agency). EHA will communicate weekly with Co-I Hughes to coordinate and monitor implementation of research and reporting to ZTWC. Co-I Hughes will coordinate activities at all other Peninsular Malaysia institutions: NPHL, the National reference laboratory for diagnostic confirmation of pathogens, will manage molecular and serological screening (BioPlex) of Orang Asli samples, and serological screening of syndromic samples from Sabah and Sarawak; the PERHILITAN molecular zoonosis laboratory will store and conduct molecular and serological screening on wildlife samples; and Universiti Putra Malaysia (UPM) Faculty of Veterinary Medicine will conduct molecular and serological screening (BioPlex) of livestock samples, should these be required. **For Sabah & Sarawak**, work will be administered through the Sabah Zoonotic Diseases Committee (SZDC), a working technical committee comprising appointed and authorized officers from Sabah State Health Dept., Department of Veterinary Services, Sabah Wildlife Dept. (SWD), Universiti Malaysia Sabah (UMS) and EHA, all of which are also committed through a signed MOU. Co-I Hughes will oversee work at all other partners in Sabah, including: the Kota Kinabalu Public Health Lab (KKPHL) for molecular screening of syndromic samples from Sabah and Sarawak; the SWD Wildlife Health and Genetic and Forensics Lab for molecular screening of Sabah wildlife samples; The Borneo Medical Health Research Center (BMHRC) for screening some Sabah wildlife and livestock samples, if required, and human syndromic samples from Sabah and Sarawak. **In Thailand** all human community and wildlife research and testing will be coordinated by co-I Wacharapluesedee from the TRC-EID center. Clinical surveillance will be overseen by senior clinical physician and co-I T. Hemachudha.

**4.1.c. Approval and release of results:** In our experience, it is critical when working in resource-poor countries, on potentially important pathogens, to strictly adhere to protocols for release of results. EID-SEARCH will liaise with existing points of contact in the Ministries of Health, Environment, and Agriculture in each our administrative areas to approve and release project findings publicly. Results from human screening will be shared with participants when they become available, as per our IRB agreements ensuring no violations to anonymize data requirements (**see Protection of Human Subjects**).

**4.2. Flexibility to extend the EID-SEARCH to new sites as needed:** The EID-SEARCH consortium partners maintain extensive working relationships with leaders in EID outbreak control, clinical investigations and research at over 50 clinics, research institutes and public health laboratories across Southeast Asia. Due to space constraints, we haven't listed each of these, nor have we solicited >50 Letters of Support for this project. However, each core EID-SEARCH partner has contacted their networks and obtained permission for inclusion in the broader goals of the EIDRC. As examples of these contacts, our core partner, the Thai Red Cross Emerging Infectious Disease Health Science Centre (TRC-EID) at Chulalongkorn University, also serves as the WHO Collaborating Centre for Research and Training on Viral Zoonoses and has ongoing research collaborations across WHO SEARO countries including Bangladesh, Bhutan, Democratic People's Republic of Korea, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, (Thailand), Timor-Leste; and has recently served as a training hub for scientists from Malaysia, Myanmar, Laos, the Philippines, and China to learn methods of wildlife sampling and diagnostic screening. Our Thai clinicians (Co-I T. Hemachudha and KP P.

Hemachudha) provide regular case consultations and clinical trainings for doctors across SEARO countries, including with Yangon General Hospital and the National Health Lab in Myanmar, 2018. To maximize leverage of this broad network, EHA has budgeted for annual meetings in SE Asia, in addition to regular smaller network meetings, with our core team and key public health experts from network labs in each of the 10 SE Asian countries. Additionally, we will set up a listserv and an internal communication network to facilitate collaboration and information exchange, including on the first reports of new disease outbreaks. Our annual and smaller network meetings will critically allow face-to-face meetings of the EID-SEARCH that will foster greater sharing of information on novel research and diagnostic approaches, pathogens that are of key pandemic potential, regions or populations at high risk of spillover, and information from the greater network on likely outbreaks of novel disease. This platform will coordinate sample sharing and diagnostic platforms and help build a rapid response to outbreaks in the region, guided by the PI, Deputy and the Executive Committee.

**4.3. Outbreak response:** EHA collaboration with expert networks around the world allows us to mobilize and enhance effective One Health response to disease emergencies (191), ranging from real-time situation updates and risk analyses to on-the-ground investigations (192-194). We will adopt management tools from Emergency Operating Center (EOCs) (195) and Incident Management Systems (IMS) (196), to shift resources where necessary to help respond to novel zoonotic outbreak events and other public health emergencies. EHA has extensive experience working with governments in low and middle income countries (LMIC) applying these principles of epidemic preparedness during outbreak responses we've been involved with under the USAID-PREDICT project. For example, at the request of the government of Bangladesh, we provided technical field and laboratory support for Nipah virus and avian influenza outbreak investigations, assisting with wildlife sampling as part of the outbreak response alongside human and domestic animal sampling. In India, we provided technical assistance in response to the Nipah virus outbreak in Kerala in 2018. Last month in Indonesia were assisted the Ministry of Health's Center for Health Laboratory in Makassar to provide technical assistance in a mysterious outbreak in a small village in South Sulawesi that killed 4 villagers and infected 72. Our network partners include the key government and govt. approved laboratories that would be directly involved in public health emergency response in their respective countries. The serological and PCR platforms that EID-SEARCH develops will be made available to the main government outbreak investigation teams for clinical work and research during the outbreak. EID-SEARCH will also offer assistance training and conducting animal sampling during an outbreak, epidemiological analysis and modeling to help identify likely reservoirs or likely pathways to spread. Technical and material support for lab, field and analytical activities during an outbreak will be provided by EHA, UNC, USU, Duke-NUS, and NEIDL, as well as in-country partners. Any clinical samples, viral isolates and sequence data will be shared among partners to promote the rapid development of new diagnostic assays, reagents, and therapeutics that can be deployed to the region or other regions as part of the larger NIH EIDRC network.

Finally, while the initial pathogen focus of our group is on CoVs, PMVs and FVs, our broad collaborative group has multidisciplinary expertise on a number of virus-host systems. For example: PI Daszak was PI on a subaward from PI Laura Kramer's U01 on Poxviruses and Flaviviruses, managing a multidisciplinary research project on West Nile virus ecology. He was also co-I on a 5-year NSF-funded project to understand West Nile virus dynamics and risk in the USA (197-201); Co-I Baric is a global leader in Norovirus research leading to the development of vaccines and therapeutics (202-205); Co-I Wang has conducted significant work on bat immunology, therapeutic, and reagent development, as well as being involved in a range of outbreak investigations, viral discovery programs and other research on a wide diversity of viral groups (206-215). Additionally, the serological and PCR-based diagnostic platforms being developed by Co-Is Wang and Broder are adaptable to other viral targets. The modeling tools developed by Co-Is Olival and Zambrana-Torrel can be used to predict the emergence and spread of diverse viral targets, including influenza, antimicrobial resistance, and vector-borne diseases (216-221). Our clinicians working in Thailand and Malaysia have a wide range of infectious disease investigations to adapt to any outbreak situation.

**4.4. Communications:** EHA will coordinate communication among all co-Is and key personnel, including:

- Multiple meetings per week with PI, Deputy Lead, Senior Program Manager (on project and task status)
- Weekly web/phone meetings between Program Manager and subawardee admin. staff
- Monthly web/phone conferences between EHA PIs and all subawardee PIs.

- Monthly web conferences between key personnel (research presentations/coordination)
- In-person Annual meetings with partner leads, key personnel at EHA and two in-person partner meetings annually between subawardees.
- Annual in-person meeting among all key personnel

**4.5. Problem identification and resolution:** Regular planning, monitoring, and evaluation meetings will be the primary mechanisms for problem identification. Minor problems (e.g. delays in sample availability or test results) will be dealt with internally through appropriate action and resolution monitored by PI Daszak and co-Principal Investigator Olival, and our Senior Program Manager. In the event of significant problems, such as prolonged poor productivity, inadequate scientific collaboration, or major disputes regarding research direction or resource allocation, EHA will assist with resolving the problem through negotiation with relevant co-PIs and consultation with the Executive Committee. Should a resolution not be forthcoming, consultation with the EIDRC-CC, additional external technical advisors, and NIH staff may be warranted.

**4.6. Adaptive management and risk mitigation:** Maintaining a timeline and meeting milestones will require strict and continuous oversight of all project phases, frequent and regularly scheduled communication, and the ability to make decisions and implement strategies. To maintain our timeline on all projects, including the EID-SEARCH, we use an adaptive management approach to continually evaluate these trade-offs, to make decisions about when iteration is appropriate and when it is necessary to move forward with current information. Our ethos is that regular, scheduled communication among all staff, partners and collaborators will go a long way towards mitigating risks, especially if the process is collaborative and transparent.

## 5. Data Management Plan

EHA will house the Data Management and Analysis (DMA) team for EID-SEARCH, led by Co-PIs Olival and Zambrana-Torreal 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 Modeling and Analytics lead for the PREDICT project (122), the Western Asia Bat Research Network (222) and EHA's Rift Valley Fever Consortium. We will leverage our experience and infrastructure from those projects to benefit the EID-SEARCH. **5.1. Project Database:** We will create a dedicated, centralized EID-SEARCH 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 paper- and tablet-based field data entry and with the Lockbox laboratory information management systems (**Section 5.2**) in place in individual partner labs. 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 EIDRC 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.

**5.2. Biological Specimen Management:** Project laboratories will use the Lockbox Laboratory Information Management System (LIMS), to manage the security, traceability, and quality of biological specimens. The LIMS will support sample barcoding at creation, tracking through transport, storage/inventory, and use via portable scanners. Lockbox supports CLIA and ISO 17025 as well as direct export to NCBI formats such as Sequence Read Archive. We will use the Lockbox LIMS application programming interface (API) to link to the central project database and associated samples with field and ecological data. We note that the project focuses on highly pathogenic viruses, including select agents; Lockbox LIMS supports sample tracking and movement compliant with US Select Agent Regulations and US Department of Commerce Pathogen Import and Export Control Regulations, and includes all necessary encryption, security, and backup protocols.

**5.3. Training:** Members of the DMA 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, and visit each partner institution and/or field team base for training workshops and analysis consultations.

**5.4. 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, and destroyed at the end of the project - as per details provided in the Clinical Management Plan and Protection of Human Subjects forms.

**5.5. Computing Resources:** EHA operates a cluster of high-performance servers for data analysis activities, as well as infrastructure to launch cloud-based computing environments (**see EHA Facilities**). Our servers host all necessary software for statistical and bioinformatics 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, and will be available to all project partners for analysis and training.

**5.6. Data and Code Sharing:** See details provided in the **Resource Sharing Plan**.

## 6. Clinical Management Plan

**6.1. Clinical site selection:** Our consortium partners have been conducting lab and human surveillance research, including during outbreaks, for >20 years and have developed strong relationships with local clinical facilities and processes in SE Asia and in LMIC globally. The plan for selection of clinical sites will begin in the same geographic regions as those identified in Aim 1 with high zoonotic viral diversity. Clinical sites will additionally serve as the catchment healthcare facilities for people in our community-based surveillance of Aim 2. We have already developed successful working relationships with the major healthcare facilities in Thailand and Malaysia and will use these established partners to rapidly gain appropriate permits and begin data collection quickly. Focusing on these EID hotspots in select biogeographic areas (see **Fig 13**) also reduces the number of additional sites needed to meet our goals within our geographic range. The capacity and capability requirements for clinical sites are fairly minimal, and include ability to enroll patients that meet the clinical case definitions of interest, collect and temporarily store biological samples, and follow standards for data management and subject protection with locked filing cabinets to store all paper records and an encrypted computer. We will be enrolling inpatient and outpatient participants within 10 days of the presentation of symptoms to increase the chance of PCR detection of viruses so we will not wait for advanced normative diagnostic tests, that are likely unavailable in the more remote sites, to be completed. We will work with clinical sites to determine the best process for project staff, in most cases supporting the time of currently hired staff at each site. We will recruit and train hospital staff in project-specific procedures including enrollment of patients, sample types to be collected, storage of samples, administration of the questionnaire, and data management plans. During the development and onboarding of new clinical sites we will assess the physical needs of the site and what supplies will be required for collection of data.

**6.2. Standardized approach, oversight, and implementation:** Management and oversight for all study sites will be undertaken by the local country coordinator with support from our Core Administrative team at EHA. Our research team has over 10 years of experience building capacity on human subjects' research and has developed training resources and materials for standardized implementation of community and clinical research and SOPs for screening, enrollment, and retention of participants. The country coordinator will conduct regular site visits to the clinical sites and annual visits to observe, monitor and evaluate the research process, and conduct follow-up training if required. Through our work with clinical sites under the USAID-PREDICT project we have developed culturally appropriate screening measures for clinical sites that do not disrupt the flow or quality of medical treatment received by the patient. This was done by working collectively with clinical staff to evaluate current procedures and patient flow at the site to determine the most efficient while minimal invasive inclusion of our study into the daily working of the clinical site. Most efficiently this was done by adding minimal basic screening questions to the current clinical intake forms, which allows the clinical research officer to quickly scan charts or logs for potential patients to enroll avoiding potential enrollees from being overlooked if staff are too busy or not on duty. Patients will be enrolled following established clinical criteria (**see Section 6.3**), samples collected and brief surveys conducted to assess the participants contact with wildlife through: 1) occupational exposures; 2) animal contact; and 3) the environment. With permission

from each clinic, and consent from participants, we will review clinical records to collect data on medical history, clinical syndromes, and patient etiology. Additionally, we will be following up with clinical participants to determine how clinical presentation differs between CoV, henipavirus, or FV in exposed and unexposed participants, as well as in the time course of illness, severity of symptoms, and type of symptoms. The country coordinator will be continually monitoring the project database to ensure we hit target sample sizes. While patient's enrollment is limited by the number of individuals presenting at hospitals, in previous research we enrolled an average of 105 patients per year, ranging from 77-244.

**6.3. Clinical cohort setup, recruitment, enrollment:** We will recruit inpatients and outpatients from clinical sites to participate in the study after initial screening to determine if they meet the clinical case definitions for 1) severe/acute respiratory illness (SARI/ARI); 2) Influenza-like illness (ILI); 3) fever of unknown origin (FUO); 4) encephalitis; 5) hemorrhagic fever, of unknown etiology or severe diarrhea with unusual presentation for symptoms to increase the chance of PCR detection of viruses. Once enrolled, biological samples will be collected by trained hospital staff. When possible, samples will be taken concurrently with samples being collected for normative diagnostics. We will collect 5mL of blood for whole blood and serum collection, samples will be aliquoted, at least one max. 500  $\mu$ L whole blood; two 500  $\mu$ L serum samples and two nasal or oropharyngeal swabs will be collected. Controls who test positive for CoVs, FVs, or Henipaviruses will be selected from the pool of participants admitted to the studies and whose samples tested negative for coronaviruses, henipavirus, or filoviruses. We will follow up 35 days after enrollment to collect an additional 5mL blood draw for collection of two 500  $\mu$ L serum samples and a standardized questionnaire supplement to collect additional data on the course of symptoms in the interim period. Following up 35 days post initial collection gives adequate time for 1) development of IgG and IgM, which occurs <28 days after onset of symptoms to evaluate the immunological progression of disease and 2) further the risk assessment of the participants to monitor contact with wildlife, people, and to assess the likely reservoir hosts to collect information to inform potential intervention programs.

**6.4. Utilization of collected data:** Sampling of wildlife and the enrollment of community surveillance participants and a clinical cohort of participants give us the ability to assess the viruses that are circulating in each of the three populations in a similar geographic region. We will use phylogenetic analysis to compare the relationship between wildlife viruses found and viruses found in human participants. Additionally, the questionnaire data will allow us to assess relative measures of human-wildlife contact that we will analyze the intensity of contact with species known to be at a higher risk for spillover against other risk to provide useful proxy information for spillover risk. The clinical cohort will be split into cases and controls. "Cases" are defined as participants whose samples tested positive for either CoV, henipavirus, or FV via PCR tests. "Controls" will be selected from the pool of participants admitted to the studies and whose samples tested negative. We will use nearest neighbor matching to pair cases demographically (e.g. age, sex, location) with controls at a 1-to-3 ratio or greater. In clinical study, we will use this procedure to determine how clinical presentation differs between virus-exposed and -unexposed participants, as well as in the time course of illness, severity of symptoms, and type of symptoms. We will model the outcomes to analyze correlation between biological results and risk factors related to high risk wildlife exposure. With this collective data set we aim to quantify and detect novel viruses likely to spillover regularly to people, are often unreported or misdiagnosed as their clinical manifestations, and are unknown to detect cryptic outbreaks causing previously 'hidden' clinical syndromes in people. Our strategy for targeted surveillance and detection of spillover and illness in at-risk human populations can be used as an 'early warning system' to conduct public health interventions and disrupt disease emergence.

**6.5. Development of reagents of value to the community.** Members of the EID-SEARCH consortium have substantial experience producing reagents, assays, and other products that are used widely by the clinical and research community, and some of which are on a pathway to commercialization. These include: PIs Daszak and Co-I Olival have produced software for analyzing the spread of novel viral agents through air travel networks; Co-I Baric has collaborated with a Norovirus surveillance collaboration with surveillance cohort at CDC and has developed therapeutics that have reached phase 2 and 3 clinical trials, He is currently working with Takeda Sanofi Pasteur on a Dengue therapeutic and with NIH on a tetravalent vaccine; Co-I Broder

developed a Hendra virus subunit vaccine that was commercially produced by Zoetis for horses and is labeled for human use under compassionate circumstances during outbreak situations.

**6.6. Potential expansion:** Expansion of the research project to other clinical sites or research areas could happen as new information is gathered, either through our research, the EID-SEARCH information network, or an outbreak being identified in the region by other organizations. If expansion is required we would rapidly shift research activities towards the clinical or community sites where the outbreak is active, using the same process we used to set up initial research locations. First, by working with local stakeholders to create a working relationship and on-boarding new clinical research staff through advanced trainings of project aims, SOPs on data collection and storage, and ethical guidelines. The network of infectious disease researchers in the community and hospital settings would allow for an accelerated response to any potential infectious disease outbreaks as staff are well trained on ethical guidelines for working with human subjects, data and specimen collection, storage, and transpiration, project staff would be on the front lines ready for the call to action. Ideally, our testing for viruses in priority viral families may lead to the discovery cryptic outbreaks before it spreads in the general public. Biological results coupled with survey data on key risk factors will provide necessary information for granular understanding of disease spread.

## 7. Statistical Analysis Plan:

**7.1. Framework:** Statistical analyses across the project will be conducted under a common Bayesian framework. These models provide a unified, probabilistic approach best-suited for estimating effect sizes in heterogeneous populations of human clinical and wildlife subjects in observational studies. Within this Bayesian framework, we will use generalized linear mixed models to estimate population prevalences and seroprevalences, and estimate the effects of demographic, occupational and environmental factors affecting these. We will use occupancy models (223) to estimate total viral species and strain diversity and completeness of sampling within the human and wildlife sub-populations, and discrete phylogeographic models to identify taxonomic and geographic centers of viral diversification. All statistical analyses will be performed reproducibly using scripted, programmatic workflows (e.g., the R and Stan languages) and maintained under source code version control (git). As with data management, the DMA team will act as trainers and consultants for exploratory data analysis, power analysis, and study design with project partners, and the EHA computing cluster will be available for partners undertaking additional or expansion studies. Power analyses, current and expansion, are performed via simulation approaches allowing planning for complex, hierarchical variation in study populations. Power analyses and specific analytical components of this study are detailed under each Specific Aim.

**7.2. Data Quality Control and Data Harmonization:** All data will be examined at entry by field and lab teams upon data entry, followed by examination by DMA team members at upload and integration, for complete de-identification, completeness, accuracy, and logical consistency. The DMA will provide field and lab teams with reports, produced automatically, of data summaries, including aggregates, distribution, detected outliers and possible mis-entries. On a regular basis (quarterly or as-needed during data collection), DMA team members will review reports with field and lab teams to identify errors and update collection and entry procedures as necessary.

**7.3. Statistical Considerations for Behavioral Questionnaires and Clinical Metadata:** The data collected from the questionnaire will be analyzed to assess the reported measures of contact for each risk group under study, related to 1) occupation and occupational exposures; 2) observed or reported interactions with priority wildlife, especially bats, rodents, and primates in/around house and other livestock animals (e.g. farming, butchering, slaughtering); 3) proximity of residence or workplace to environments of increased risks (e.g. nearby bat roosts); 4) working or regular visitor to animal markets; 5) self-reported ILI/SARI clinical diagnosis or symptoms in the past 12 months and lifetime. Specific measures we are interested in are the proportion of respondents indicating they consume wildlife, where wildlife is obtained for consumption, have hunted wildlife, butchered or slaughtered live animals, seen wildlife in their homes, been bitten or scratched by animals, etc. Comparisons of measures of exposure contacts and types between men and women, children and adults, different study regions will be conducted in order to explore the occupational, environmental, and demographic factors (gender, age, socioeconomic status (SES)) that influence contact with animals and to determine who is

most at-risk. Statistical analysis will be employed to identify differences between groups with a 95% probability of detecting a difference. Measures of contact related to different activities within specific groups will be compared to determine the activities that put groups at most risk. As appropriate multivariate analysis (e.g. ordinary linear regression, logistic regression, non-normal distributions of outcome, least absolute shrinkage and selection operator (LASSO) regression, etc.) will be utilized to evaluate the relationship between the outcome variables, positive biological results (PCR or serology) key measures of contact and the factors that influence frequency and types of human-animal contact.

### 8. Project Milestones and Timelines

**8.1 Milestones: End of Year 1: Aim 1:** Sample targeting locations, species (for wildlife), sample size justifications completed for whole project and reported to in-country teams; Sample testing, viral isolation, NGS, glycoprotein sequencing begun for all archival and some newly-collected samples; *in vitro* work begun; host-pathogen dynamic analyses; animal model work begun. **Aim 2:** Target human community populations identified and sample sizes calculated for some sites in each country; Community data collection, serological testing and RT-PCR testing begun; first epidemiological analyses of data begin in last quarter. **Aim 3:** Clinical cohort selection underway; clinical enrollment, data collection and sample analysis begun. First Annual meeting in last quarter. First publications submitted by end of year, summary overview papers or reviews.

**End of Year 2: Aim 1:** No sample targeting or sample size justification analyses needed. All other aspects underway **Aim 2:** All aspects underway **Aim 3:** All sub-aims underway. Second Annual meeting in last quarter. Further 2 publications submitted by end of year, including first data papers.

**End of Year 3: Aim 1:** No sample targeting or sample size justification analyses needed. All other aspects underway **Aim 2:** All aspects underway **Aim 3:** All sub-aims underway. Third Annual meeting in last quarter. Further 3 publications submitted by end of year, largely data papers.

**End of Year 4: Aim 1:** No sample targeting or sample size justification analyses needed. All other aspects underway **Aim 2:** All aspects underway. Receptor binding work completed. **Aim 3:** No further cohort selection required; all other sub-aims underway. Fourth Annual meeting in last quarter. 3 further publications submitted, including first papers analyzing risk factors, pathogenic potential of novel viruses submitted.

**End of Year 5: Aim 1:** No sample targeting or sample size justification analyses needed. No receptor binding assays continuing. Serological and PCR testing completed end of 2<sup>nd</sup> quarter. Glycoprotein, *in vitro* and *in vivo* analyses, analysis of viral risk continue to end of project. **Aim 2:** No further community targeting or sample size work. Community data collection completed at end of 2<sup>nd</sup> quarter. All other aspects continue to end of project **Aim 3:** All sub-aims underway. Final Annual meeting in last quarter. Further 3 publications submitted.

### 8.2. Timeline:

ACTIVITIES	YEAR 1				YEAR 2				YEAR 3				YEAR 4				YEAR 5			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
<b>AIM 1</b>																				
1.4.a. sampling targets																				
1.4.b. sample size justifications																				
1.4.c. sample collection & testing																				
1.4.d. NGS																				
1.4.e. sequencing Spike GP																				
1.5.a. human cell infection																				
1.5.b. receptor binding																				
1.5.c. host-pathogen dynamics																				
1.5.d. viral strain prioritization																				
1.5.e. animal models																				
<b>AIM 2</b>																				
2.4 target population & sample sizes																				
2.5 community data collection																				
2.6.a serological testing																				
2.6.b RT-PCR testing																				
2.6.c virus characterization																				
2.7 epidemiological analysis																				
<b>AIM 3</b>																				
3.4.a cohort selection																				
3.4.b clinic enrollment & follow-up																				
3.4.c clinical data collection																				
3.5 sample testing																				
3.6 risk characterization																				
annual meeting																				

## PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved

Yes  No

Is the Project Exempt from Federal regulations?

Yes  No

Exemption Number

1  2  3  4  5  6  7  8

Other Requested Information



**Human Subject Studies**

Study#	Study Title	Clinical Trial?
<u>1</u>	Understanding Risk of Zoonotic Virus Emergence in EID Hotspots of Southeast Asia	No

### Section 1 - Basic Information (Study 1)

1.1. Study Title \*

Understanding Risk of Zoonotic Virus Emergence in EID Hotspots of Southeast Asia

1.2. Is this study exempt from Federal Regulations \*

Yes  No

1.3. Exemption Number

1  2  3  4  5  6  7  8

1.4. Clinical Trial Questionnaire \*

1.4.a. Does the study involve human participants?

Yes  No

1.4.b. Are the participants prospectively assigned to an intervention?

Yes  No

1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?

Yes  No

1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?

Yes  No

1.5. Provide the ClinicalTrials.gov Identifier (e.g. NCT87654321) for this trial, if applicable

## Section 2 - Study Population Characteristics (Study 1)

### 2.1. Conditions or Focus of Study

- Humans living in geographic hotspot areas/close contact with wild animals

### 2.2. Eligibility Criteria

#### ELIGIBILITY CRITERIA

Participants to be enrolled in this study will be individuals from Thailand (Ratchaburi and Chonburi provinces), Peninsular Malaysia, Sabah Malaysia, or Sarawak # 12 years old living or working around wildlife habitats (e.g. bat caves/roosts), those who hunt wildlife, work with wildlife or livestock farming, transportation, selling, or slaughtering wildlife or visiting or working in high-risk sites (e.g. wildlife markets) who meet the inclusion criteria outlined below. Study sites are prioritized based on the hotspot geographic areas described in Aim 1, according to ecological and epidemiological conditions associated with a high risk for the coronaviruses, henipaviruses, filoviruses spillover.

Research participants will be enrolled in two settings:

1. Community - We aim to enroll and collect biological samples and survey responses individuals' living, working, or visiting targeted high-risk communities (as defined above) who have close contact with wildlife, specifically bats, rodents, non-human primates, with a range of exposures to these animals. Enrolled research participants will be asked to provide biological samples and complete a questionnaire that is designed to obtain detailed information into wildlife contact frequency and exposures related to: 1) occupation and occupational exposures; 2) observed or reported interactions with priority wildlife, especially bats, in/around house and other livestock animals (e.g. farming, butchering, slaughtering); 3) proximity of residence or work place to environments of increased risks, e.g. nearby bat roosts; 4) working or regular visitor to animal markets; 5) self-reported symptoms relating to a) severe/acute respiratory illness (SARI/ARI); b) Influenza-like illness (ILI); c) fever of unknown origin (FUO); d) encephalitis; or e) hemorrhagic fever; or f) diarrhea in combination with any of the previously mentioned illnesses within the 12 months and lifetime.

Additional inclusion criteria:

- Adults (18 years of age or older) who provide informed consent
- Children aged 12-17 years of age who provide assent along with an accompanying parent or guardian who is able to provide informed consent and
- Pregnant women will be considered eligible for inclusion

Exclusion criteria:

- Adults (18 years of age or older) who are unable to provide informed consent, including individuals with physiologically or medically induced cognitive impairments
- Individuals under 12 years of age
- Children without an accompanying parent or guardian who is able to provide informed consent, or a child 12-17 years old unable or unwilling to provide assent or children who are wards of the state
- Prisoners

2. Hospital - Both out-patients and in-patients at clinics or hospitals presenting with clinically defined symptoms of 1) severe/acute respiratory illness (SARI/ARI); 2) Influenza-like illness (ILI); 3) fever of unknown origin (FUO); 4) encephalitis; or 5) hemorrhagic fever; or 6) diarrhea in combination with any of the previously mentioned illnesses of unknown etiology. Biological samples will be collected from the patients and the patient, will complete a questionnaire. We will follow up with these participants 35 days after enrollment to collect another biological sample to assess the development of IgG/IgM and collect additional data on the course of symptoms in the interim period.

Additional inclusion criteria:

- Adults (18 years of age or greater) who provide informed consent
- Children aged 12-17 years of age who provide assent along with an accompanying parent or guardian who is able to provide informed consent and
- Pregnant women will be considered eligible for inclusion

Exclusion criteria:

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

### 2.3. Age Limits

Min Age: 12 Years

Max Age: N/A (No limit)

### 2.4. Inclusion of Women, Minorities, and Children

Inclusion\_of\_Women\_Min\_Children\_FINAL.pdf

Contact PD/PI: DASZAK, PETER

2.5. Recruitment and Retention Plan	Section_2_Attn_Recruitment_Retention_Plan_FINAL.pdf
2.6. Recruitment Status	Not yet recruiting
2.7. Study Timeline	Section_2_Attn_Study_Timeline_FINAL.pdf
2.8. Enrollment of First Subject	09/01/2020                      Anticipated

## INCLUSION OF WOMEN AND MINORITIES:

This study will enroll men and women, including pregnant women, as study participants. Subjects will be enrolled in this study without regard to ethnicity.

Women who volunteer to participate are not at an increased risk based on pregnancy status and are at the same exposure risk as non-pregnant women. Every effort will be made to protect the privacy, dignity, and well-being of all study participants especially special populations who participate in this study.

Individuals in sub-sites in selected geographic hotspot regions will be the primary mechanism for identifying subjects. We will make every effort to have men and women equally represented in this study and no individuals will be excluded based on ethnicity.

- **At community sites**, living or working around wildlife habitats (e.g. bat caves/roosts), who hunt wildlife, work with wildlife or livestock farming, transportation, selling, or slaughtering wildlife in the surveyed areas will be the primary criteria for identifying participants in community.
- **At clinic sites**, only patients who present at the healthcare facility who meet the clinical case definition of 1) severe/acute respiratory illness (SARI/ARI); 2) Influenza-like illness (ILI); 3) fever of unknown origin (FUO); 4) encephalitis; or 5) hemorrhagic fever; or 6) diarrhea in combination with any of the previously mentioned illnesses of unknown etiology will be recruited for this study, and no patients will be excluded based on ethnicity or gender.

## INCLUSION OF CHILDREN:

Children aged 12–17 years will be included in this study, and there will be no maximum age restriction for adults, at both community and clinical sites

- Previous clinic-based studies have shown that children are one of the major populations who present to healthcare facilities with severe/acute respiratory illness (SARI/ARI), Influenza-like illness (ILI), or fever of unknown origin (FUO). Our behavioral study in Thailand and Malaysia also suggested the close contact with wild animals among children in the study regions via activities of animal hunting, trade, or butchering.
- Children aged 12 years or older are post-primary school in Thailand and Malaysia and are able to comprehend and respond to the questionnaire autonomously which increases the reliability of responses. We will not enroll children aged 12-17 years without an accompanying parent or guardian who is able to provide informed consent, or a child aged 12-17 who is unable to or unwilling to provide assent.
- Children under age 12 in target communities are mainly school children who have very limited exposures to wild animals under the scenarios of interest to the study, and ethically we do not want to collect or enroll participants without strong scientific need for inclusion. We will not enroll children who are wards of the state
- Every effort will be made to protect the privacy, dignity, and well-being of children who participate in this study. Our in-country human research team are well-trained medical doctors and researchers who have extensive experience working with children, as well as their parents, at both community and clinical settings. Prior to the start of human subject research activities, all research staff will be CITI-trained and further trained on conducting ethical human subject research training including a module on the special considerations for working with children

regarding risk and coercion. Enrollment of children will be monitored and annually reported to the IRB.

## RECRUITMENT AND RETENTION PLAN

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

Community visits will begin with discussions and meetings with local authorities and community leaders to introduce ourselves and our project, and when appropriate following approval from local authorities, the study team will post flyers to inform the community when the team will be speaking about enrollment and later coming back to enroll interested individuals. Attending this “town hall” style meeting will be completely voluntary and based on our experience, those interested are likely to attend. Although local authorities may be present to introduce the study team members, they will not be involved in the recruitment and/or consent of the participants for the study. Individuals will be clearly informed during the recruitment process that their participation in the study is voluntary. If research visits or recruitment events are held at a workplace individuals choice of involvement will not impact their employment, nor will information discussed be shared with employers. With local permissions and accompanied by local community leaders, district health officers, or authorities the study team members will engage in community town halls and ‘walkabouts’ during which they will discuss study details, dates, times, and locations for enrollment and participation in the study.

Participation in the study will be strictly voluntary and will require signed informed consent for all participants and signed assent for participants aged 12-17 along with parent or guardian consent. During the enrollment process interested individuals will be given a consent form and research staff will read the consent form to potential participants. Together they will review the consent form and study staff will explain details of the study including: why they were selected, what the study procedures are and what will be expected from them, potential risks and benefits of their participation, that their participation is completely voluntary, and that they can withdraw their participation at any time. After reviewing the consent form individuals will be given as much time as needed to ask questions. At that time if individuals wish to participate they will sign two copies of the consent form and it will be countersigned by the research staff, with a copy given to the participant for their records. Included in the consent form is the contact information for local research staff and a local IRB contact for participants if they have questions in the future. Responses will be kept strictly confidential. Measures will be taken to assure the privacy, dignity, and respect of each participant. Ethical human subjects research methods will be a focus in all training of research staff, we will emphasize the importance of avoiding coercion during enrollment and protecting the privacy of participants.

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

**Clinic-based recruitment:** Patients eligible for enrollment will be identified during standard intake procedures or from overnight intake logs, or in the emergency room, ward, or intensive care unit of each participating clinic or hospital by collaborating clinic staff. Employed staff at each location will identify potential participants meeting the clinical case definition of 1) severe/acute respiratory illness (SARI/ARI); 2) Influenza-like illness (ILI); 3) fever of unknown origin (FUO); 4) encephalitis; 5) hemorrhagic fever; or 6) diarrhea in combination with any of the previously mentioned illnesses of unknown etiology. Patients will be screened for eligibility according to the inclusion/exclusion criteria based on available clinical information and clinical presentation.

We have set a minimum target enrollment sample size to detect live virus in patients at each hospital assuming a population prevalence of 1% with a 95% probability. We will work with the local institution review board to determine the maximum enrollment of patients without undue burden on the population. However, in larger

Program Director/Principal Investigator: Daszak, Peter

tertiary healthcare centers where many cases fitting study inclusion are expected or are being enrolled we will regularly evaluate enrollment logs to be sure we are prepared to collect samples throughout the length of the sample collection timeline as to not miss a change in circulating virus. If we need to control the number of patients being enrolled at a hospital interval sampling will be implemented by selecting every Nth case of those individuals who meet enrollment criteria. The interval will be determined in collaboration with the local research staff and implementing partners based on an evaluation of the enrolled participants to date and expected number of cases presenting at the site within a given year in order to best meet study design and sample size criteria and stay IRB compliant. In terms of retention, we will express our gratitude to subjects for their participation and discuss the research importance of the follow-up data collection. Nonetheless, we expect to have an approximate 40% loss to follow up and have included this in our sample size calculations.



## **STUDY TIMELINE**

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

This will be an ongoing five-year project from the time of award.

- We anticipate obtaining all required IRB approvals and local permissions in the first 6 months of projects;
- We will start human subject enrollment at community and clinical sites in Year 0.5 at the earliest, and enrollment will continue through Year 5, to be completed by the conclusion of the project;
- Human sample testing will start in Year 0.5 at the earliest, with completion of analyses by the end of the award.

**Inclusion Enrollment Reports**

IER ID#	Enrollment Location Type	Enrollment Location
Study 1, IER 1	Foreign	Ratchaburi and Chonburi provinces in Thailand; Peninsular Malaysia, Sabah Malaysia, and Sarawak in Malaysia

### Inclusion Enrollment Report 1

Using an Existing Dataset or Resource\* :       Yes       No

Enrollment Location Type\* :       Domestic       Foreign

Enrollment Country(ies):      MYS: MALAYSIA, THA: THAILAND

Enrollment Location(s):      Ratchaburi and Chonburi provinces in Thailand; Peninsular Malaysia, Sabah Malaysia, and Sarawak in Malaysia

Comments:

#### Planned

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	4150	4150	0	0	8300
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	0	0	0	0
White	0	0	0	0	0
More than One Race	0	0	0	0	0
<b>Total</b>	<b>4150</b>	<b>4150</b>	<b>0</b>	<b>0</b>	<b>8300</b>

#### Cumulative (Actual)

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/Not Reported	Female	Male	Unknown/Not Reported	Female	Male	Unknown/Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	1217	867	5	0	0	0	0	0	0	2089
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	0	0	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	0	0
More than One Race	0	0	0	0	0	0	0	0	0	0
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
<b>Total</b>	<b>1217</b>	<b>867</b>	<b>5</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>2089</b>

### Section 3 - Protection and Monitoring Plans (Study 1)

3.1. Protection of Human Subjects

Section\_3\_Protection\_Human\_Subjects\_FINAL.pdf

3.2. Is this a multi-site study that will use the same protocol to conduct non-exempt human subjects research at more than one domestic site?

Yes     No     N/A

If yes, describe the single IRB plan

Section\_3\_sIRB\_plan\_FINAL.pdf

3.3. Data and Safety Monitoring Plan

3.4. Will a Data and Safety Monitoring Board be appointed for this study?

Yes     No

3.5. Overall structure of the study team

## PROTECTION OF HUMAN SUBJECTS:

### 1. Risks to Human Subjects

#### 1.1 Human Subjects Involvement, Characteristics, and Design

This project is a study of human spillover and exposure to animal coronaviruses, henipaviruses, and filoviruses in Southeast Asia, with active sample collection in Thailand and Malaysia and testing of archived samples in Singapore. As there is substantial evidence that these viruses likely spillover regularly to people, are often unreported or misdiagnosed and thus underestimated; and this targeted surveillance and detection of spillover and illness in at-risk human populations can be used as an 'early warning system' to conduct public health interventions and disrupt disease emergence. Subjects will be enrolled on a voluntary basis and informed consent will be obtained from all participants and assent from all participants aged 12-17. Consenting participants will provide biological samples for PCR or serological testing and complete a questionnaire to collect information on wildlife exposures and frequency. Subjects will be individuals: 1) who are highly exposed to wildlife, specifically bats, rodents, and non-human primates, in community settings, through hunting, butchering, or general handling within the context of their living or working environments ( $\geq 18$  years old); and 2) patients admitted to hospitals and clinics presenting with disease symptoms of clinically-defined 1) severe/acute respiratory illness (SARI/ARI); 2) Influenza-like illness (ILI); 3) fever of unknown origin (FUO); 4) encephalitis; or 5) hemorrhagic fever; or 6) diarrhea in combination with any of the previously mentioned illnesses of unknown etiology.

The study population will be selected from the subnational geographic hotspot regions listed in Aim 1. We will enroll participants from: 1) communities at 4 sub-sites from each of the regions of interest in Ratchaburi (Thailand), Chonburi (Thailand), Peninsular Malaysia, Sabah Malaysia, and Sarawak, 175 individuals from each of sub-site in the 5 regions will be collected and pooled across the region for a total of 700 participants per region, allowing us to make region-level comparisons of differing effects, enrolling a total of 3,500 participants; and 2) patients from the selected 2 town-level level clinics and 2 provincial-level hospitals in each of the geographic regions of Thailand, Peninsular Malaysia, Sabah and Sarawak, we will enroll a minimum of 300 participants per clinic or hospital, for a total of 16 healthcare facilities and 4,800 total clinical participants. This will yield 2,880 participants that will be available for follow-up blood sampling assuming for an estimated 40% loss from follow-up. The community and clinical sites are further defined in Specific Aims 2 and 3.

There are no data to suggest a gender or ethnic bias for coronaviruses, henipaviruses, and filoviruses exposure or infection, therefore individuals will be enrolled based on exposure criteria alone and individuals will not be excluded based on ethnicity or gender. We will also monitor sampling enrollment to ensure equal representation of sex, demographic, and socio-economic factors in each community site.

#### 1.2 Sources of Materials

Biological samples to be collected and tested for coronaviruses, henipaviruses, and filoviruses include whole blood, serum, and nasal/oropharyngeal swabs. Samples will be collected by locally trained medical personnel and a questionnaire will be administered by research or collaborating staff from the local hospitals and clinics.

In community sites, whole blood samples will be collected from participants one time during the data collection period during Years 2-5 of the study. The whole blood samples will be aliquoted into at least one max. 500  $\mu$ L whole blood and two 500  $\mu$ L serum samples. Samples will be tested for coronaviruses, henipaviruses, and filoviruses using developed ELISA by consortium partners. For participants who report the symptoms relating to 1) severe/acute respiratory illness (SARI/ARI); 2) Influenza-like illness (ILI); 3) fever of unknown origin (FUO); 4) encephalitis; or 5) hemorrhagic fever; or 6) diarrhea in combination with any of the previously mentioned illnesses of unknown etiology within the last 10 days, an additional sample type will be collected, nasal or oropharyngeal swabs (2x). These samples will be marked for additional PCR-based assays to identify presence of known and novel coronaviruses, henipaviruses and

filoviruses, and for isolation and biological characterization of potential pathogens if PCR results are positive.

In clinic sites, both whole blood samples and nasal/oropharyngeal swabs will be collected at enrollment, whole blood samples will be aliquoted into at least one max. 500 µL whole blood and two 500 µL serum samples. Samples will be tested for coronaviruses, henipaviruses, and filoviruses using consensus PCR (cPCR). We will follow up 35 days after enrollment to collect an additional blood sample of 5mL to be separated and aliquoted into a minimum of two 500 µL serum samples that will be serological tested with the developed ELISA assay.

All blood samples will be kept frozen for future harvesting of Peripheral Blood Mononuclear Cells (PBMCs) for those study participants whose serum tests are positive for emerging viral pathogens. These will be used for harvesting polyclonal and monoclonal antibodies as potential therapeutics.

During data collection a standardized questionnaire will be administered to both community and clinic participants. This survey will collect data on exposure type and frequency with wildlife focusing on: 1) occupation and occupational exposures; 2) observed or reported interactions with wildlife, especially bats, rodents, and non-human primates, in/around house; 3) proximity of residence or workplace to environments of increased risks, e.g. nearby bat roosts; 4) working or regular visitor to animal markets; 5) self-reported ILI/SARI clinical diagnosis or symptoms in the past 12 months. During the follow-up with clinic participants a standardized questionnaire supplement will be administered to collect additional data on the course of symptoms in the interim period. All electronic data will be password protected, and all hardcopy files and biological samples will be stored in secure storage facilities. All consent forms and participant logs will be stored separately from research data in locked filing cabinets.

### **1.3 Potential Risks**

The potential risks to study participants as a result of study participation are minimal. The biological specimen will be collected by locally certified healthcare professionals proficient in phlebotomy techniques, the volume of blood being collected is within normal safety limits and the swab sample is not overly invasive. The questionnaire will be designed to assess exposure risk and may ask personal questions, however, administration will be conducted privately and confidentially to protect individuals' personal health and exposure information. There may be some stress or discomfort for participants who are informed that they have been exposed to a zoonotic virus, to reduce this counseling will be available and options for future medical care will be included in the discussion with the health official or physician reporting results back to individuals.

## **2. Adequacy of Protection against Risks**

### **2.1 Recruitment and Informed Consent**

Potential study participants at each site will be recruited after obtaining local permissions and support from local community leaders, district health officers, and/or authorities the study team members will engage in community town hall meetings and 'walkabouts' during which they will discuss study details, dates, times, and locations for enrollment and participation in the study for individuals who wish volunteer to participate. The team will be trained on conducting ethical human subjects research before the commencement of data collection and enrollment of participants. This will include the importance of avoiding coercion during enrollment, protecting the privacy of participants, how to effectively communicate the research objectives, what is being asked of participants, any risks or benefits to participation, with sufficient support to be able to address any questions that potential participants may have. Training will also include a module on special populations for advanced training on working with minors during human subjects research. During the enrollment process interested individuals will be given a consent form in the local language and research staff will read the consent form to potential participants, via an interpreter in local dialects if necessary. Together they will review the consent form and study staff will explain details of the study including: why they were selected, what the study procedures are and what will be expected from them, potential risks and benefits of their participation, that their participation is completely voluntary, and that they can withdraw their participation at any time. After reviewing the consent form individuals will be given as much time as needed to

ask questions. At that time if individuals wish to participate they will sign two copies of the consent form and it will be countersigned by the research staff, with a copy given to the participant for their records. Contact details for local research staff member, a local IRB contact, and project PI will be provided to all subjects in the consent form to answer any future questions or requests for withdrawal participants may have.

## **2.2 Protection against Risks**

Biological sample collection: collection of whole blood samples and nasal or oropharyngeal swab samples pose minimal risk to subjects. The potential complications associated with whole blood draw include pain and/or hematoma at the site of venipuncture. Nasal or oropharyngeal swab sample collection may cause minor irritation at the time of collection. To protect against and minimize potential complications, all biological sampling will be done by a locally trained and certified healthcare professional and/or clinic staff, and the sample collection sites will be monitored according to existing health facility protocols.

Risk factor questionnaire survey: potential risks associated with the administration of the questionnaire may be discomfort or concern providing responses related to wild animal contact or consumption if practices are taboo or prohibited by local laws. To minimize this risk, questionnaire data will be collected in a strictly confidential manner. The questionnaire will be conducted in private, ensuring that others cannot overhear participant responses and a barrier will be used or created so that no other individuals can view the participants. Depending on the location, this could be a private room, behind a building or fence, or behind a line of trees, obstructing view so that confidentiality may be maintained. The interview team will take care to pair interviewers and participants by sex to the best of their ability to increase the level of comfort of the participant and the team will ensure the privacy and confidentiality of response data. Children aged 12-17 will not be interviewed in the absence of a parent or guardian. Every effort will be made to ensure the privacy, dignity, and well-being of children and adults who participate in this study. In addition, identifying information will not be linked to responses, and data will be stored in secure, password protected files or locked secure storage facilities.

Participants may feel some stress or discomfort if informed that they have been exposed to a known or novel zoonotic virus, to reduce this counseling will be available and options for future medical care will be included in the discussion with the health official or physician reporting results back to individuals. Additionally, we will provide participating hospitals, clinicians, and community leaders with information and background data on relevant zoonotic viruses.

## **3. Potential Benefits to Subjects and Others**

There are no measurable benefits to the individual study participants enrolled in this study. There may be secondary benefits including receiving a physical exam/health check from a medical officer at the time of enrollment or advanced non-diagnostic testing assays that add clarity medical history for clinic participants. There are also benefits to the community and regional healthcare providers understand the risk of zoonotic infections among high-risk populations. At the conclusion of the study, we will deliver an educational workshop reporting study findings that will be open to both study and non-study participants, describing the health benefits of using PPE and hand-washing during animal handling activities throughout the day, as well as to share other prevention interventions that emerge from the research data.

## **4. The Importance of Knowledge to be Gained**

There are valuable potential benefits to the general public from the knowledge to be gained from this study. One key benefit of this study to the community an understanding the risk of zoonotic spillover events among high-risk populations. This strategy for targeted surveillance and detection of spillover and illness in at-risk human populations can be used as an 'early warning system' to conduct public health interventions and disrupt disease emergence. As well as share information with communities on practices that could reduce exposure and related health risks such as the avoidance of particular animals or the need for PPE and extra care when handling wildlife may substantially reduce the risk zoonotic pathogen transmission.

Knowledge gained will also increase understanding of the conditions and human activities associated with the introduction of zoonotic infections into human populations, which may have implications for disease control more broadly.



**SINGLE INSTITUTIONAL REVIEW BOARD (sIRB)**

In compliance with the NIH Policy on the use of a single IRB of record for multi-site research EcoHealth Alliance will prepare, submit, and work the institutional review board that follows the ethical standards set forth by the HHS regulations at 45 CFR 46. Once this single IRB is approved in the US it will function as the IRB of record and will be relied on at all planned sites and any future sites.

We are currently anticipating working with HummingbirdIRB to serve as the IRB of record for all study sites. All of our local research partners, partner institutions, and study staff will rely on the IRB protocol that is approved at the IRB of record for all planned and future sites where data collection will occur. All data collection (biological and questionnaire) procedures and protocols and consent processes will be conducted using the same protocols outlined in the approved IRB of record and consistent for all location sites. The approved protocol at IRB of record will serve as the foundation for all locally submitted IRB packages in all partner countries, Thailand, Malaysia, and potential Singapore for inclusion of archived samples for testing.

EcoHealth Alliance will submit for IRB approval and maintain all records, and annually manage and submit for continuing review approvals at the IRB of record. Additionally, EcoHealth Alliance will manage the authorization and reliance agreements between partners and implement the communication plan. Each partner implementing human subjects research in Thailand, Malaysia, and Singapore will maintain regular communication with scheduled updates to the EcoHealth Alliance point of contact on enrollment and recruitment numbers, breakdown of enrollment of special populations and report any adverse events within 8 hours if not sooner.

Prior to commencing study enrollment or sample testing, the partner organization that is managing human subjects enrollment in Thailand, Malaysia, and Singapore will sign a reliance agreement that will acknowledge the role of the IRB of record and responsibilities of the participating institutional partners.

**Section 4 - Protocol Synopsis (Study 1)**

4.1. Brief Summary

4.2. Study Design

4.2.a. Narrative Study Description

4.2.b. Primary Purpose

4.2.c. Interventions

Type	Name	Description
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4.2.d. Study Phase

Is this an NIH-defined Phase III Clinical Trial?  Yes  No

4.2.e. Intervention Model

4.2.f. Masking  Yes  No

Participant  Care Provider  Investigator  Outcomes Assessor

4.2.g. Allocation

4.3. Outcome Measures

Type	Name	Time Frame	Brief Description
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4.4. Statistical Design and Power

4.5. Subject Participation Duration

4.6. Will the study use an FDA-regulated intervention?  Yes  No

4.6.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/ Investigational Device Exemption (IDE) status

4.7. Dissemination Plan

**Delayed Onset Studies**

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