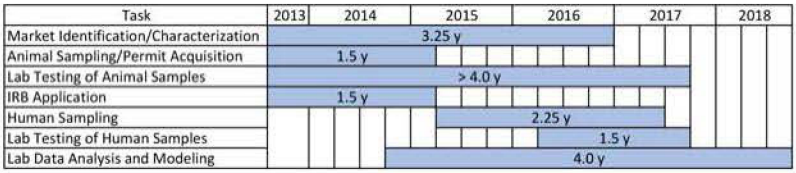
C3d) Humanized mouse *in vivo* infection experiments: To evaluate pathogenicity of bat-CoVs we will perform *in vivo* infection experiments in humanized mice modified to carry human ACE2 or DPP4 gene in the Wuhan Institute of Virology BSL-3 animal facility. We will passage isolated bat-CoVs in permissive cells twice, administer a specific inoculum (e.g. $1x10^6$ TCID50) to intranasally or intraperitoneally. Mouse body temperature will be monitored with implanted microchips (LifeChip Bio-thermo, Destron Fearing), and mice will be weighed and observed for clinical signs of illness daily. Dead or moribund mice will be euthanized, organs harvested and sectioned. Live animals will be euthanized at three weeks post-inoculation and organs harvested. We will test for neutralizing antibodies against bat-CoVs on days 10, 15 and 21 pi. We will collect nasal washes, oral swabs, and rectal swabs, and urine every two days and quantify virus using qRT-PCR. We will conduct routine histology, immunohistochemistry, qRT-PCR, and virus isolation on tissues. This work will provide information about viral pathogenicity, tissue tropism, transmission route, and infection symptom.

C3e) Binding affinity assay: The recombinant S proteins and receptor molecules (e.g. ACE2 or DPP4) will be expressed in insect cells or eukaryotic cells. Octet RED platform (ForteBio, Menlo Park, CA)) will be used to perform binding affinity kinetics experiments. Streptavidin-coated sensor tips from Fortebio will be used to capture biotinylated S protein onto the surface of the sensor. After reaching baseline, sensors will be moved to the association step containing indicated concentrations of wild or mutant receptor molecules diluted with kinetics buffer for 30 min and then dissociated for 30 min at 25°C. Binding affinity will be determined by collecting the dissociation constants KD, Kon (association-rate), and Koff (dissociation-rate) determined by fitting binding chromatogram data with the Octet®User Software.

C3f) Potential pitfalls and solutions: Through our targeted sampling in China, we may only identify a small portion of the huge diversity of bat-CoVs in bat populations. To resolve this, we plan to expand our sampling locations to include samples from across SE Asia and improve our detection methods targeting more virus sequences. We will also synthesize the S genes based on the published data for viruses we do not obtain. Virus isolation may be a big challenge for this specific aim. In our previous work, we have isolated a number of novel bat viruses including adenovirus, reovirus and SARS-like CoV and have refined and optimized our methods for virus isolation. We will also attempt to construct additional bat cell lines which are lacking interferon response or over expressing the receptor molecules and more susceptible for virus infection to increase isolation success.

D. TIMELINE & MANAGEMENT PLAN:



This project will take 5 years to complete. The initial phase will involve filing the IRB application, identifying sampling sites, and conducting animal sampling and testing. Mid-project efforts will involve initial human sampling, analyses of lab results and production of models. The final phase will involve testing human and wildlife samples and analyses and modeling to maximize results. **Project Management:** Funds will be managed via subcontracts originating with EcoHealth Alliance, which is an A133 (low risk)-audited 501 (c) 3 organization specializing in international research on emerging diseases. PI Daszak will oversee all aspects of the project management. He is an experienced manager, with over 15 years of federally-funded research experience. Prof. Shi, based at the Wuhan Institute of Virology, will oversee all laboratory testing and analyses. Prof. Shuyi Zhang will manage field sampling work. EcoHealth Alliance staff will manage all modeling and analytical approaches (Aims 1 & 2). Communication will be via monthly video-conferences using EHA's NIH ARRA-funded video-conference facility. Travel budget has been requested to enable regular face-to-face meetings for all key staff.