Preventing emergence and spillover of bat viruses in high-risk global hotspots

STATEMENT OF WORK

July 17th 2018

Milestones by Task

CIES: Cary Institute of Ecosystem Studies; **CSU**: Colorado State University; **Cornell**: Cornell University; **GU**: Griffith University; **JH**: Johns Hopkins University; **MSU: Montana State University**; **PSU**: Penn State University; **RML**: Rocky Mountain Laboratories; **TTU**: Texas Tech University; **UCB**: University of California, Berkeley; **UCLA**: University of California, Los Angeles; **Cambridge**: University of Cambridge.

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TA1

COLLECT AND ANALYZE FIELD SAMPLES

Task 11.01, Data collection: longitudinal sampling of wild bat populations and a captive population. Cambridge, GU, JH, and UCB, with assistance from MSU and TTU, will sample multiple bat populations longitudinally in multiple locations and ship retrospective bat samples to RML or local laboratory for analyses (11.03).

Task 11.02, Data collection: retrospective analysis of bat samples. Cambridge, GU, JH, and UCB will identify, locate, and ship retrospective bat samples to RML or local laboratory for analyses (11.03).

Task 11.03, Lab: screening, metagenomics to identify virus and quasispecies. RML and Cambridge or local laboratory will screen and sequence samples from bats; create a list of sequences that have spilled over from bats to other species; and select sequences for genotype-phenotype modeling.

Task 11.12, Lab: screening retrospective samples from human/domestic livestock hosts. Cambridge, JH, and UCB will identify, locate, and ship retrospective human/livestock samples to RML or local laboratory for analysis; RML or local laboratory will screen samples and create a list of sequences that have spilled over from bats to other species.

Milestones

Australia (GU will do field collection and RML or local laboratory will do sequencing):

- Establish field sites and train field teams (6mths)
- Sample up to 40 bats in 4 bat colonies monthly for 2 years (12mths, 24mths)
- Respond to spillover events or viral pulses within the study area by sampling adaptively until prevalence decreases (12mths, 24mths)
- PCR on all samples for Hendra virus (30mths)
- Sequence all positive samples available (36mths)
- Analyze 1000 retrospective bat samples for henipaviruses (24mths).

Bangladesh (JH will do field collection and in-country PCR; RML will do sequencing):

- Sample up to 40 bats in 4 colonies monthly for 2 years (24mths)
- Respond to spillover events or viral pulses by sampling adaptively until prevalence decreases (24mths)
- PCR on samples for Nipah virus (30mths)
- Sequence all positive samples available (36mths)
- Analyze retrospective bat samples for henipaviruses (24mths).

Ghana (Cambridge will do field collection and laboratory analyses, with some help from RML):

- Locate retrospective human and animal samples suitable for testing and establish sequencing pipeline (6 months)
- Sample up to 120 bats per quarter in 3 colonies, perform PCR testing on the first batches and send positive sample for sequencing (12 months)
- Update sampling effort in bat colonies for year 2 based on 12 months result, for PCR and sequencing, with up to 500 bats to be caught in year 2 (24mths)
- Sample bats in the captive colony every 3 months (24mths)
- Sequence all positive samples available (36mths)

Madagascar (UCB will do field collection and PCR, and RML or local laboratory will do sequencing):

- Establish field sites and train field teams (12mths)
- Sample up to 30 bats in 3 colonies monthly for 2 years (12 mths, 24mths)
- Respond to viral pulses by sampling adaptively until prevalence decreases (12 mth, 24mths)
- Analyze 700 retrospective bat samples for henipaviruses (12mths)
- PCR on samples from bats at Institut Pasteur de Madagascar (30mths)
- Sequence all positive samples available (36mths)

Historic humans and livestock samples (JH, Cambridge, UCB):

- Identify and ship historic samples to RML or local laboratory (6mths)
- PCR on samples from humans and livestock (12mths)
- Sequencing of all positive samples available (18mths)

IDENTIFY HOST IMMUNE SIGNATURES

Task 11.04 Lab: identify host immune and stress signatures in wild bats and in a captive feeding trial. MSU, with help from CSU will measure bat immune signatures. TTU will measure bat stress signatures and nutritional status. A captive feeding trial will be conducted in Ghana (Cambridge), or alternatively, if a natural nutritional stress event occurs in Australia during Phase I, this trial will be conducted in Australia (GU).

Milestones
Immunology on samples from Australia (MSU):

- Validate and optimize tests for each bat species (6mths)
- Immunological markers such as IgG and IgA, biomarkers of cell damage, gene expression of antiviral & proinflammatory proteins, and microbial killing assays for 400 samples (30mths)

Immunology on samples from Ghana (Cambridge):

- Titrate antibodies against Henipaviruses in sera from all PCR-positive bats and a sample of up to 1000 PCR-negative bats, from wild and captive colonies (24 mths).

Stress signatures on samples from Australia (TTU):

- Test up to 720 hair and fecal samples for cortisol (30mths)
- Develop methodology to use bioelectrical impedance analysis to measure body condition of bats (12mths)
- Measure body condition of 400 bats (24mths)

Captive feeding trial (Cambridge, GU)

- Conduct experimental diet manipulation to test the effect of nutritional status on immune state and viral shedding (30mths)

COLLECT ENVIRONMENTAL, ECOLOGICAL, and RESERVOIR HOST DATA

Task 11.10 Remote sensing data, longitudinal short-term weather and long-term climate data, land cover change, human population data, bat movement data. PSU will identify environmental drivers of shedding in Australia and detect large bat colonies through remote sensing. TTU will implement bat telemetry.

Milestones
Remote sensing (PSU):

- Collect data on weather, climate, and land cover change in Australia (24mths)
- Collect data on human population dynamics across space (local/region), time (seasonal/decadal) (24mths)

Bat movement data (TTU):

- Deploy GPS tracking devices on bats in resident and nomadic colonies in Australia (12mths, half deployed; 24mths all deployed)
- Collect, collate, and analyze bat movement data (36mths)

CREATE GENOTYPE-PHENOTYPE MAPS FOR HENIPAVIRUS QUASISPECIES BASED ON *IN VITRO* AND *IN VIVO* WORK

Task 11.13, Lab: in vitro experiments to assess jump potential of quasispecies to new hosts. Cornell and RML will quantify determinants of zoonotic potential for henipavirus strains and quasispecies.

Milestones

Cloning (Cornell; 24 mths):

- Prioritize sequences for 20 F and G pairs to be analyzed for receptor binding and membrane fusion (24mths)
- Synthesize and clone sequences for 20 F and G gene pairs in pCAGGS plasmids (24mths)
- Grow plasmids in bacteria for 20 pairs F and G pairs (24mths)

Receptor binding and membrane fusion assays (Cornell, with help from RML; year 2, 12mths)

- Complete receptor binding assays for 20 G sequences (year 2, 12 months)
- Complete membrane fusion assays in 3 cell lines (human, bat and pig) (year 2, 12 months)

Molecular docking with *in silico* with *in vitro* measurements (RML, with help from Cornell):

- Perform molecular docking analyses (24mths)

Task 11.08, Lab: amplification and transmission dynamics of quasispecies *in vitro* and *in vivo*. RML, with help from CSU, will undertake in vivo experiments to measure phenotypes of henipavirus strains.

Milestones

In vitro and *in vivo* work (RML):

- Use cell culture experiments to analyze growth kinetics of henipaviruses (12mths)
- Develop hamster model for infection experiments (24mths)
- Conduct infection experiments in hamster model to measure infection, shedding, & QS in model hosts (24mths)
- Compare pathogenicity and transmission characteristics in hamster studies with historic studies done by RML (30mths)
- Obtain lung samples at peak virus replication and deep sequence these samples to study QS and selective pressures in a dead-end host (30mths)
- Develop bat models for henipavirus strains with highly pathogenic characteristics in the dead-end host model (36mths)
- Conduct infection experiments and measure infection and shedding in bats (36mths)
- Upon sufficient shedding, conduct contact transmission experiments (36mths)

- Analyze inoculated vs. transmitted virus populations by deep sequencing and identify potential transmissible QS (36mths)
- Analyze QS by established long-read PCR NSG methods (ongoing 42mths)

ANALYZE DATA

Task 11.05, Data analysis: statistical analysis of field data, lab data, environmental and ecological data, and bioinformatics NGS data. Provide statistical support and manage database for project.

Milestones

Data analysis and support (MSU):

- Develop a database structure, system and procedures for providing access to data, and a data visualization platform to facilitate information sharing across tasks and institutions (12mths)
- Clean and check data as it arrives (ongoing over 24mths)
- Graphically visualize and share incoming data for full team (ongoing over 24mths)
- Manage database, analyze data as appropriate, and provide statistical support to the team (ongoing 42mths)

Specific analyses to support other Tasks:

- Use statistical modeling to investigate and quantify links among nutritional status (TTU), stress signatures (TTU), immune status (MSU) and viral shedding (GU/RML/local laboratory) in *wild Australian bats* (30 months)
- Use statistical modeling to investigate and quantify links among nutritional status (Cambridge), stress signatures (MSU), immune status (Cambridge) and viral shedding (Cambridge) *in captive bats* (42 months)

DEVELOP MODELS

Task 11.06, Stochastic models of within- and between-host virus dynamics in bats. Cambridge, with help from GU, will perform stochastic modeling of within and between host virus dynamics in bats.

Milestones

Modeling (Cambridge, GU):

- Develop models of virus transmission within bat populations using prior knowledge from each location (12mths)
- Develop generic models of within-host virus dynamics that incorporate measurable components of the bat immune system (12mths)
- Validate and refine within- and between-host models of virus dynamics in bats using data collected in each field site and laboratory (36mths)

Task 11.15, Mechanistic mathematical modeling of viral fitness within humans, bats, and other host species, iterated with lab studies. UCLA will assemble genotype-to-phenotype maps for reservoir and spillover host species.

Milestones
Viral fitness modeling (UCLA):

- Develop mechanistic model of viral life cycle within cells (12mths)
- Integrate molecular, virologic, cell culture, and animal experiment data (24mths)
- Compare fitness predictions from *in silico* vs *in vitro* data (36mths)
- Integrate models and lab data to establish empirical relations between viral traits and fitness (42mths)

Task 11.09, Phylodynamic models of quasispecies dynamics within bat populations and between host species. MSU, with help from Cambridge and UCLA, will perform phylodynamic modeling of henipaviruses in bat populations.

Milestones
Phylodynamic modeling (MSU, Cambridge, UCLA):

- Formulate model framework to link viral genetics to transmission dynamics (12mths)
- Create models of within- and between-host selection in bat populations (24mths)

Task 12.02, Multi-scale models of zoonotic transmission from bats to humans to predict quasispecies expansions and pulses of excretion. Cambridge, with help from MSU, UCLA and GU, will develop a multi-scale mechanistic modeling framework for pathogen spillover.

Milestones
Multi-scale modeling (Cambridge, MSU, UCLA, GU):

- Develop baseline tools to relate spillover modeling framework from Plowright et al. to field data (12mths)
- Adapt spillover modeling framework from Plowright et al. to henipavirus contexts; identify key challenges to operationalize (18mths)
- Integrate bat virus transmission dynamics, environmental data. and viral fitness models (30mths)
- Develop an integrative model of bat virus spillover that is operationalized to predict probability of spillover at a spatial and temporal scale relevant for intervention (42mths)
- Perform a two-step validation of models:
    - Internal validation of the fitting methods: using simulated data generated by our candidate models, we will infer the parameter values and check the accuracy and precision of the fitting method (ongoing over 42mths)
    - External validation: we will exclude parts of the data iteratively, fit the models to the remaining dataset and check that it predicts correct values for the missing data (ongoing over 42mths)

Task 11.16, Machine learning to ID virus, reservoir traits, zoonotic risk. CIES will perform machine learning analyses to prioritize surveillance by identifying combinations of bat traits and environmental factors that predict spillover.

Milestones
Machine learning analyses (CIES): (all activities below are ongoing over 36mths)

- Collate and pre-process multiple data streams from field teams (environmental data; ecological data on bat populations; data on human ecology)
- Engineer features; impute bat trait data; tune hyperparameters for selected machine learning algorithm; execute cross-validation and target shuffling procedures to diagnose and correct overfitting; produce trait profiles of bat species predicted to be henipavirus positive (first predictions at 6mths).
- Repeat procedures above for models at the ecoregion and country scales (ongoing over 36mths)
- Combine species-level predictions with environmental and human ecological features from the Australian system (i.e., corresponding with viral shedding pulses in local bat populations, satellite imagery on seasonal human population densities, fruiting phenology, climate induced stress). Identify bat species that present the greatest spillover risk to humans, and measurable features that best predict viral shedding (ongoing over 24mths)
- Incorporate data on viral shedding events and conduct machine learning on viral PCR data to identify detectable predictors of viral shedding (Phase 2)
- Assess features corresponding to parameters in a multiscale mechanistic model of viral shedding and provide machine learning support of features to be included in multi-scale models of viral dynamics (e.g., engineering features, estimating parameters impacting viral shedding) (ongoing over 18 months in Phase 2)

TA2
DEMONSTRATE PROOF OF CONCEPT FOR AN ECOLOGICAL INTERVENTION FOR SPILLOVER

Task 22.03, Proof-of-concept for preemption through strategic ecological interventions. GU, with help from TTU and PSU, will do preliminary studies to develop the proof-of-concept demonstration of an ecological intervention to stop spillover. GU, MSU, TTU, Cambridge, CIES, CSU, PSU, RML will all contribute to investigating links between nutritional stress and virus shedding (above).

Milestones.
Demonstrate that bats move from urban roosts to flowering events in native forests (GU, with help from TTU)

- Establish methodology for using movement data to validate bats moving from urban roosts to native forests (6mths)
- Acquire movement data from existing and projected sources (18mths)
- Analyse movement data (24mths).

Demonstrate that bats locate and feed in regenerated habitat

- Develop experimental design and field methods to test use of regenerated forest as feeding habitat by bats (6mths)
- Establish field sites for testing use of regenerated forest as feeding habitat and commence field sampling (12mths)
- Sample up to 30 paired regeneration sites and remnant native habitat (control) sites for feeding bats (18mths)
- Analyse feeding data (24mths)

DEMONSTRATE PROOF OF CONCEPT, FEASIBILITY, AND SCALABILITY OF CHAD/VSV VACCINATION

Task 22.02, Proof-of-concept demonstration of ChAd/VSV vaccination feasibility and scalability of ChAd/VSV vaccination in bats. RML will develop and test a scalable vectored vaccine for target henipaviruses in bats. RML, with help from Cambridge, will assess the feasibility and scalability of the vaccine in bats.

Milestones

Vaccine development (RML):

- Design novel vaccines based on TA1
- Test by comparing measures of protection with historic hamster models (12mths)
- Test the effectiveness of the vaccines against novel henipaviruses (24mths)
- Demonstrate reduced probability of virus transmission among bats and among bats and recipient host species *in vivo* (42mths)
- Quantify scalability of ChAd/VSV vaccination in captive bats in Ghana (42mths)

TRANSITION PLAN

MSU and RML will develop the research transition plan.

Milestones

- Work with the MSU technology transfer infrastructure and personnel, and with the CEPI program to develop partnerships with vaccine manufacturers (30mths)
- Developed an inter-institutional agreement to enable the transfer of our discoveries to industry for commercialization (36mths)