

VERTEBRATE ANIMALS:

1. Detailed description of animal use.

Work with vertebrate animals will be conducted at Wuhan University at the School of Medicine in Wuhan, China and the University of North Carolina, Chapel Hill, USA.

Capture and sampling techniques for all wild animals (bats) described in this study have been previously approved by multiple Institutional Animal Care and Use Committees (IACUCs) for projects led by EcoHealth Alliance. These institutions include: UC Davis IACUC (Mazet and Epstein; UC Davis 15898; current); and The Cummings School of Veterinary Medicine at Tufts University (Olival and Epstein, current), Animal Welfare Assurance (#A4059-01) on file with the Office of Laboratory Animal Welfare at the National Institutes of Health. We have prepared a draft IACUC application for this project, and will submit it within 1 month of the project's start date (to Tufts University) to minimize delays in beginning Year 1 field sampling.

Experimental work using humanized mice will be conducted at the Center for Animal Experiment Biosafety 3 lab of Wuhan University at the School of Medicine in Wuhan, China and the University of North Carolina at Chapel Hill, the Institute for Pathogen Biology. The Wuhan laboratory is AAALAC accredited and has both an Institutional Biosafety Committee and an Institutional Animal Care and Use Committee. We will submit our protocols for IACUC approval should this proposal be funded. Conditions for animal use are described below. Both laboratories (Wuhan and UNC) have Internal Biosafety Committees and are accredited BSL-2 and BSL 3 laboratories. Animals will be housed in a BSL-3 facility and will be under the care of a full-time veterinarian. All experimental work using infectious material will be conducted under appropriate biosafety standards. Disposal of hazardous materials will be conducted according to the institutional biosafety regulations.

Note: The majority of wild bats captured and sampled will be done using non-destructive, techniques. In a small number of instances (~ 2 bats per species for previously unsampled *Rhinolophus* sp.), where intestine and lung tissue is required to establish cell lines, animals will be humanely euthanized and a necropsy performed according to accepted protocols (see euthanasia section)

Bat capture. Free-ranging bats will be captured using either a mist net or harp trap. The net system is manned by two people during the entire capture period, and bats are removed from the net as soon as they become entangled to minimize stress and prevent injury. In the Co-PI's (Dr. Olival) experience, a maximum of 20-30 bats can be safely held and processed by a team of three people per trapping period. Duration of trapping will depend on the capture rate. Bats are placed into a pillowcase or small cloth bag and hung from a branch or post until samples are collected. Bats are held for a maximum of six hours (typically less than 3 hours), and released after sampling.

Laboratory mice. Lab mice will be sourced commercially by the Wuhan Center for Animal Experiment at Wuhan University.

Sample Collection:

Bats: Bats will be manually restrained during sampling. Depending on the species and size of bat, swabs will be taken from the oropharynx, urogenital tract, and rectum. Fresh feces will be collected if available, in which case a rectal swab will not be collected. Blood will be collected from fruit bats either from the cephalic vein or from the radial artery or vein using a 25 gauge needle and 1cc syringe. Blood