

## **VERTEBRATE ANIMALS:**

### **1. Detailed description of animal use.**

#### **All work with vertebrate animals will be conducted in China.**

Capture and sampling techniques for all wild animals described in this study have been previously approved by UC Davis IACUC (Mazet and Epstein; UC Davis 15898; current).

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. The Center is AAALAC accredited and has both an Institutional Biosafety Committee and an Institutional Animal Care and Use Committee. Animals will be housed in a BSL-3 facility and will be under the care of a full-time veterinarian. We will submit our protocols for IACUC approval should this proposal be funded. Conditions for animal use are described below.

**Note: The majority of wild animals captured and sampled will be done using non-destructive, techniques. In a small number of instances (~ 2 bats per species), 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. Epstein) 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.

**Wild rodent capture.** Free-ranging rodents will be captured through pit traps and box traps; captive rodents, including resident free-ranging wild rats/rodents in markets, will be manually captured or captured through traps. Traps will be checked a minimum of once daily in the morning. If adverse weather (extreme heat, rain) is expected or researchers are working in areas where predation is common, traps will be checked more frequently, and closed during the adverse weather. Handling of rodents will involve morphometric measurements. Captive and wild rodent sampling procedures (including anesthesia if necessary), will involve manual restraint, venipuncture, mucosal swabs, fecal, urine, and external parasite collection. Following capture, small animals will be restrained with a fine mesh bag to minimize entanglement, taking precautions to ensure the animals are not traumatized by the hoop of the net or through net removal. Larger rodents will be restrained for sampling in specialized squeeze-cages, allowing adjustments appropriate to the size of the animal. Squeeze-cages consist of a wooden frame with a plasticized wire bottom and a Plexiglas shield used to press the animal, while ensuring visible communication between the field veterinarian and the animal. Once squeezed, a rod is inserted to keep the plastic shield in place. The box is then inverted, allowing sampling to be conducted through the open wire bottom and abdomen of the animal when the animal is safely immobilized. Anesthesia for small rodents will be conducted using plastic tubes, with the animals transferred directly from the traps to the tubes containing a cotton swab soaked in ether, isoflurane, or methoxyflurane for anesthetic induction. For larger rodents, chemical restraint and anesthesia (ketamine alone, or ketamine combined with xylazine) will be applied either through the squeeze cages by syringe if applicable.

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

**Sample Collection.** Bats will be manually restrained during sampling.

**Bats:** 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 will be collected from bats weighing less than 100g according to published techniques (126).

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