

human receptors. We'll infect them with cultured bat coronaviruses and determine which organs become infected and whether these mice are capable of shedding infectious virus. Humanized mice will be genetically modified to carry human ACE2 gene will be used to evaluate pathogenesis of CoVs. We cannot anticipate exactly how many viruses we will find that are candidates for experimental models, however will likely identify approximately 5-6 bat SARSr-CoV strains that will be used for mouse infection experiments. We will use 15-20 adult mice per virus strain, and therefore will require a maximum of 120 mice over the study period.

3. Provide information on veterinary care. For wild caught animals, there is no specific veterinary care that is appropriate, nor will clinical veterinary facilities be available. Animals that are injured during the capture or sampling process will be assessed by an experienced team leader, and if the animal is determined to be unlikely to survive if released, it shall be euthanized humanely (see euthanasia section). Animals will be released within hours of capture.

Laboratory mice will be housed in BSL-3 small animal facilities at the Center for Animal Experiment at Wuhan University and University of North Carolina at Chapel Hill, the Institute for Pathogen Biology. Experimental animals will be regularly monitored by experienced staff and a supervising veterinarian. The animal facility operates 24 hours a day and has full-time veterinarians on staff. All animals will be provided with food and water ad libitum and will otherwise receive standard care.

4. Procedures for ensuring animal comfort, lack of distress, pain, or injury: Wild bats will not be held longer than 6 hours during the sampling process. Co-PI Olival has extensive experience in capture, anesthesia, and sampling wildlife, especially bats. In our team's experience, bats tolerate the described procedure well. Mist nets will be attended continuously during capture periods, and bats will be extracted from the net as soon as they become entangled. This will minimize stress and injury from entanglement. Bats will be placed individually in cotton bags and hung from tree branches while awaiting processing and during recovery. The bags are sufficiently porous as to allow for ventilation and are designed for bat capture. The enclosed environment seems to calm the bats, as they do not struggle once inside, but they hang quietly – this is a standard and accepted practice in the bat research world and best way to minimize stress to the animal. Animals will be monitored by a veterinarian or experienced field team member during all stages of capture, processing, and release. Animals will be kept in a cool place while in the pillowcases.

The procedures used in this experiment (blood draw, nasal, oral, and rectal swabs) are minimally invasive. Mice that show signs of morbidity post-infection will be examined and euthanized according to AVMA standards (see below).

5. Euthanasia: In the event of injury to an animal that results in pain and suffering, and reasonable veterinary care is unavailable, the animal will be euthanized by a veterinarian or trained field team member using ketamine injected intramuscularly 37.5mg/kg and sodium pentobarbital injected intravenously at a dose of 1.0ml per 5kg injected intravenously. This protocol is in accordance with the AVMA euthanasia report (2007). Any animal that is euthanized using a chemical agent will be disposed such that it will not be permitted to enter the food supply either through markets or hunting.