

PROTOCOL OF LONG-TERM MONITORING OF SMALL MAMMALS AND ZOOONOTIC DISEASE AGENTS¹

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¹ *adapted from the Centers for Disease Control and Prevention / University of New Mexico protocol
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1. SITE SELECTION

Sites for long-term ecological studies should be selected based on prior knowledge of suitably large target mammal populations, presence of viral antibody in these populations, and the security of locations from human interference. Other considerations should be proximity to roads, a weather station, and a field station for processing animals. A detailed map of the web site should be prepared, and should include exact latitude, longitude, and altitude (using Global Positioning System equipment if available, or from high quality topographic maps), references to permanent landmarks, and dominant vegetation. Using this map, the trapping web should be divided into sectors of approximately uniform vegetation (ideally all webs at each site should be located in uniform habitat) and a Physiognomy Data Sheet should be used to describe the condition of, and any changes in the vegetation each trapping period.

2. PERMANENT WEB PLACEMENT

Three replicate sampling areas will be established at each site. Each sampling area consists of a small mammal trapping "web", each with an effective trapping area of approximately 7 hectares. Each trapping web and the trap stations within them must be established by precise measurement to insure accurate data on rodent density and movements throughout the monitoring period. Each web contains twelve 100m transects radiating from a central point in a pattern which resembles the spokes of a bicycle wheel when viewed from above (Figure 1). Each web includes

148 Sherman and 24 Tomahawk live traps. Twelve traps are placed along each of the radiating spokes, the first four at 5 m intervals and the remaining eight at 10 m intervals. Four traps are placed around the central point. Each trap station is marked with one piece of rebar, each with a metal tag numbered from 1 to 145, with trap station number 1 at the north facing spoke and radiating out to trap station number 12. Each spoke follows in a clockwise fashion with 30° between successive spokes. Trap station number 145 includes the four traps placed in the center of the web. Two Tomahawk traps are placed along each spoke, one at the end and one in the middle, six stations from the end. The webs should be separated by a distance of at least 500 m. Two of the webs will be designated sampling webs, where virus activity within rodent populations will be monitored by taking monthly blood and oral swab-samples from captured rodents. The third web, designated as a "control" web, will be used only to monitor rodent population parameters and to assess the effects of sampling on rodent survivorship.

3. TRAPPING SCHEDULES

Long-term trapping sites will be visited monthly (or another pre-determined period) for at least 5 years with each site operated for three consecutive nights close to the new moon. Field teams of at least three technicians will load the vehicle(s) with equipment on Monday, travel to the trapping site and set up the webs. Traps will be set out and baited (rolled oats or horse feed) in the afternoon. In cold weather, polyfill (best if precipitation is likely) or cotton nestlets should be placed in each

trap to provide nesting material and extra bait with added peanut butter should be used. Traps should be checked early the following morning, especially if traps are exposed to the morning sun. Protective clothing, including a long-sleeved shirt and leather or latex gloves should be worn while checking traps. Each trap with a capture should be marked with the web number and trap station with a permanent marker or pencil, and each capture individually double bagged to reduce the possibility of infecting personnel or other rodents from the web. Captured animals must be kept out of the sun and transported to the processing area as quickly as possible. After technicians are suited up, the bags should be opened to allow air to circulate. If possible, captured animals should be transported to the processing site in the back of a pickup truck or other compartment isolated from passenger sections. After processing, and releasing animals, all traps on the web should be checked and rebaited as necessary and trapping will continue for the remaining nights. Traps can remain open during the day, but should be checked around noon and in the evening to remove any diurnal captures. These captures should be processed the same day if possible. Otherwise, they should be left overnight with some moist food, such as apple, carrot, or potato chunks, and processed the following day. The field team will return to base after processing on the final day. Non-field tasks will include specimen curation and storage, data entry, and replenishment of supplies.

4. ANIMAL PROCESSING

The processing station should be located in an isolated area, away from humans, livestock, or other animals. Protective clothing including latex gloves, surgeons gowns, and Powered Air-Purifying Personal Respirators (PAPR's) or half-face respirators with HEPA filters and goggles, should be worn. If outdoors, technicians should sit with the wind at their backs. Vehicles and supplies should be located upwind, and captured rodents downwind (in the shade). Cryotubes for specimens from the sampling web should be prepared and preprinted labels (containing unique accession number, date, and sample type) attached prior to processing. After donning protective equipment, begin processing animals on the control web by removing a trap containing a captured rodent from the double plastic bag and shake the animal into a

clear plastic bag without anesthesia. The following data should be recorded on rodent data sheet (enclosure 3): accession number, date, web, trap station, tag number, fate, species, sex, age, mass, reproductive condition, and wounding data. The animal should then be returned to the original trap for later release. Captures from the sampling webs should be shaken into a plastic bag containing cotton or gauze soaked with Halothane (15%) and mineral oil (85%) or other proven anesthetic. The halothane-mineral oil mixture soaked material should be contained in a tea strainer or other porous device which will allow inhalation of the anesthesia, yet can be wiped off with a disinfectant (70% ETOH) between animals to prevent cross-infection of rodents. Animals captured in Tomahawk traps can be anesthetized by placing the entire trap into a small trash bag with anesthesia-soaked gauze or cotton or by transferring the animal to a small plastic bag using leather gloves. The deeply anesthetized animals (e.g. slowed breathing, lack of response to foot pinching) should be removed from the bag and placed on a clean surface (e.g. white paper towel) for processing. Standard (total length, tail, right-hind foot, ear, and mass) measurements and reproductive data will then be recorded. Oral swabs will be taken with dacron-tipped applicators. The applicators should be cut with scissors at the level of the dacron and inserted into 0.5 ml of virus medium (200 ml heat inactivated fetal bovine serum, 800 ml Phosphate buffered Saline, 20 ml Penicillin and Streptomycin, and 1 ml Fungizone, for 1000 ml of media) in a 2 ml cryotube. Whole blood should be taken into a second cryotube by heparinized capillary tube from the retro-orbital sinus. If the animal is a new capture (not previously caught during any trapping session) it will be marked with a uniquely numbered ear tag and/or implantable chip. The animal should then be placed in its original trap and allowed to recover fully from the effects of the anesthesia prior to release. Animals recaptured a second or third time during a trapping session should be released after noting only tag number, species, and sex. Animals from all webs should then be returned and released at the trap station of capture. A clean, baited trap should then be placed at the trap station and the dirty trap should be returned to the processing station for decontamination. Contaminated traps should be submerged in a 5-gal bucket of 10% clorox bleach for at least 10 minutes and then in a second and

third bucket containing rinse water before setting in the sun to dry. Dirt and fecal material in/on traps can be removed with a toilet brush. Heavy rubber gloves should be worn while handling traps to avoid damaging latex gloves on sharp trap surfaces. When finished processing, researchers should remove protective clothing and equipment and wash with disinfectant soap and water.

5. SPECIMEN PROCESSING AND SHIPPING

Whole bloods, oral swabs, and any ectoparasites which might have been collected should be placed in labeled cryovials and stored in liquid N₂ or on dry ice before placing at -70° C for long-term storage. Animals which die in the trap or during processing should be assigned an accession number, processed as described in the survey protocol and shipped to the archival museum you are collaborating with. A museum quality tag containing the unique accession number, date, and mass should be attached to the right hind foot of the animal. Frozen specimens should be sorted by sample type and packed, in numerical order, into specimen boxes. Cardboard shipping tubes may be used as a replacement for specimen boxes. Boxes should then be double bagged in large size, ziplock freezer bags. Absorbent packing material (or paper towel) capable of soaking up any fluids should also be added to the bottom of the cooler. Boxes should be placed into a styrofoam shipping cooler and packed with sufficient dry ice to last a minimum of

four days (approximately ~25 lbs). Shipping coolers should not be over-filled with boxes or tubes as this may preclude the adequate addition of dry ice. Fiber tape should be placed around the top of the container and the cooler sealed in a cardboard shipping box. The box should be clearly labeled with the shipping destination and the words "DIAGNOSTIC SPECIMENS" before shipping by "Next-Day" Federal Express. Each shipment should contain a packing list and a computer disk with packing information. This information can be a subset of the data collected on the rodent data sheet and should include: unique accession number, date, state, web number, species, and sample type. After each 3-day trapping session, all frozen specimens should be forwarded, on dry ice, to the serology testing or frozen tissue archiving institution you are collaborating with.

6. DATA PROCESSING

Data on each rodent capture (whether samples are obtained or not) will be recorded on a standardized data collection form. These data will be entered after each trapping session into a master Excel or Access data file. Copies of updated data files containing the new monthly records will be forwarded to the CDC with the blood and swab specimens. Further analyses including calculation of absolute densities using program DISTANCE ® can then be performed.