Nasal Lavage Collection in Tortoises

1. Evaluate for the presence of clinical signs using established data sheet.

2. Clean head/nares:
   a. If very dirty, first rinse with water.
   b. If nares are dirty, use alcohol soaked gauze/cotton ball to wipe off nares, face, and chin. Allow to air dry.
   c. If significant nasal discharge, first take an aspirate of the discharge and place directly in 1 ml media aliquot. Then clean head & nares as above and perform flush.

3. Prepare flush material:
   a. Pull up 10 cc of sterile 0.9% NaCl (saline) using a 10cc syringe and large gauge needle (i.e. 18-20 ga.). Smaller volumes may be used for subadult tortoises (approx. 6 cc saline) and juvenile tortoises (approx. 2-4 cc).
   b. Use a 22 ga. IV catheter to flush the nares. First remove the needle stylet from the IV catheter. Then remove the needle from the syringe and attach the plastic catheter to the 10 cc syringe without touching the distal tip of the catheter.

4. Restrain the tortoise and perform the flush:
   a. Have an assistant gently hold and stabilize the tortoise’s head with one hand. When grasping the tortoise, make sure to hold the head behind the jaw at the neck to minimize the chance for causing trauma to the head. Pressure is applied to the ventral aspect of the chin to push the tongue dorsally into the choanae. This will decrease the chance of aspiration of the saline or the tortoise swallowing the saline as the flush is performed.
   b. Either the person restraining the tortoise or the person performing the flush can hold an opened, sterile collection cup (i.e. urine cup) under the tortoise’s chin. Try to minimize the amount you touch the tortoise with the cup in order to keep the cup sterile and minimize sample contamination.
   c. Gently advance the catheter into the tortoise’s nare. Typically, the catheter is only advanced 2-4 millimeters. Flush half the volume of the saline (5cc for adults) into one nare and have your assistant catch the fluid in the collection cup. Sometimes the tortoise will pool the flush in its oral cavity if pressure is not maintained on the tongue between the jaws. Fluid will often be expelled through the other nare and sometimes through the mouth.
   d. Repeat the procedure in the other nare, flushing with the remaining volume (5cc for adults).
   e. Occasionally, you may notice a small amount of blood in the collected fluid. Gentle advancement of the catheter only a short distance will help to minimize this.
   f. For hatchlings or very small tortoises: In 2 separate 1cc syringes, draw up 0.5 cc – 1 cc sterile saline or SP-4 media. Grasp the head and apply dorsal...
pressure to the tongue as described above. Place the syringe (no needle attached) directly over the nare and flush media/saline in and out of each nare. Do not flush the entire volume. Leave at least 0.1 cc remaining in the syringe as typically only a very small amount of fluid is recovered and the remaining volume will be needed to flush that fluid out of the syringe. Put media into a screw-cap tube or saline directly into the media aliquot tube.

5. Label the collection cup/tube with the tortoise identification #, date collected, study site, and person submitting the sample. If available, add SP4 medium or sterile 5% bovine serum albumin to collection cup while in the field (1cc media into approx. 10cc saline). Store the sample on ice when in the field. If samples will be shipped within 3 days, store samples in a refrigerator. If samples will be held longer, transfer them to a freezer as soon as possible. As with the serum samples, it is best to use a freezer that does NOT have an automatic defrost cycle and if you plan to store the samples for an extended period of time, a -80 C deep freezer should be used.

6. Ship samples on dry ice if possible. If not, use multiple ice packs to ensure that samples stay cold.

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