

DIAGNOSTIC SAMPLING AND OTHER PROCEDURES WITH TURTLES AND TORTOISES

Kevin Wright, DVM
 Arizona Exotic Animal Hospital, LLC
 Mesa, AZ

GETTING STARTED

It is important to recognize that you can perform many of the same diagnostic procedures with a tortoise that you can with a dog or cat—just because the patient has scales and a hard shell doesn't mean you forget basic clinical techniques for working up a medical problem. What diagnostic samples are important varies based on the case you are working up and may include: 1) blood, serum, and plasma; 2) bacterial, fungal, and viral cultures; 3) feces; 4) urine; 5) cytology (fine needle aspirates, tracheal wash, touch preps, etc.); 6) biopsy samples; 7) external parasites; and 8) photographs of lesions.

Although some tortoises submit readily to common diagnostic procedures such as venipuncture and cloacal washes, a recalcitrant chelonian that retracts fully into its shell is all but impossible to sample without sedation or anesthesia. With these chelonian patients you are faced with a Catch 22 situation—you would like to have some physiological data such as blood work before proceeding with sedation anesthesia, yet in order to get the blood

sample you need to chemically immobilize the tortoise. Before you proceed, you must explain to the client why you need the samples and what the risks are of sedating a chelonian to obtain these samples. It is an Arizona state law that such a written estimate have a signed consent before performing any services on an animal but your state may have different requirements. I insist that the client sign a written treatment plan (that includes an estimate of costs) as well as a separate sedation/anesthesia approval form before going ahead with any chemical immobilization.

SEDATION AND ANESTHESIA

It is important to have a tortoise at the correct core body temperature (typically at least 84 to 86°F) for immobilization to be predictable. If the core body temperature is much cooler, drug uptake and distribution may be slowed and yield inconsistent results such as delayed induction, insufficient analgesia and muscle relaxation, and prolonged recovery. Warm water bottles and safe surgical-quality heating pads can be used to provide warmth to a tortoise undergoing an immobilization.

At Arizona Exotic Animal Hospital, most of our chelonian patients are African spurred tortoises (better known as sulcatus), leopard tortoises, and Sonoran desert tortoises. Table 1 details some of the common anesthetic protocols we use.

Table 1. Sedation and Anesthesia Protocols for Chelonians

Ketamine 10 mg/kg IM Butorphanol 0.5 mg/kg IM	May give additional 5 mg/kg ketamine and 0.5 mg/kg butorphanol if sedation insufficient after 20–30 minutes	Light to deep anesthesia achieved and useful for many procedures such as placing esophagostomy tube. Intubate and use sevoflurane for longer procedures. Our most commonly used protocol
Butorphanol 0.5–1.0 mg/kg IM Metacam 0.2 mg/kg IM		Good moderate sedation for tortoises under 2 kg. Useful for venipuncture, tracheal washes, placing intraosseous catheters, cloacal flushes, and other diagnostic procedures.
Propofol 5–10 mg/kg IV	Delivered by subcarapacial vein	It is extremely difficult to hit a vein on a retracted sulcata. However, it is very easy to give this via jugular vein in desert tortoises.
Medetomidine 0.1–0.5 mg/kg IM	May be given with 5–10 mg/kg ketamine IM.	Has worked exceptionally well on some sulcatus over 20 kg. Use equal volume of atipamizole for reversal. One leopard tortoise induced with medetomidine died even with one dose of reversal. Intubation and respiratory support often needed.
Sevoflurane 4–5% in induction chamber	Leave tortoise in chamber for 3–5 minutes after full relaxation is apparent.	Works extremely well for small tortoises (under 200 g) for minor procedures. Provides 5–20 minutes of light anesthesia; longer and deeper anesthesia if tortoise is left in chamber longer. Isoflurane does not work well—tortoises hold their breath when they smell isoflurane but do not seem to do so with sevoflurane.

DIAGNOSTIC SAMPLING

Blood

Blood samples may be extremely useful to assess a tortoise's health through leukocyte differentials, complete blood counts, plasma or serum chemistries, and serology. Chelonian blood may coagulate, if collection is slow, particularly if the tortoise has an inflammatory disease. For this reason, I like to heparinize the syringe (and butterfly catheter if one is used) before collection but this does impact the staining characteristics of blood cells and renders the sample unusable for blood culture. It may also impact other diagnostic tests. Each diagnostic lab has different requirements for sample processing so check with your lab to determine their requirements before obtaining the sample.

The jugular vein is the preferred site of collection due to the large quantity of blood that can be collected rapidly, obviating the need for an anticoagulant in the syringe. The jugular vein often rolls if a needle larger than 25-gauge is used for venipuncture. Most Sonoran desert tortoises and redfoot tortoises can be handled for jugular venipuncture without sedation. I have an assistant hold the tortoise in right lateral recumbency with the forelegs pulled back while I hold the head in my left hand and gently extend the neck until it is straight. If the tortoise is tilted so that the head is below the body, it may help fill the jugular and make it easier to see. I may be able to hold off the jugular with my left index finger or I may have the assistant do that with a free finger. I like to insert the needle with the bevel facing up and I may or may not bend the needle slightly depending on how much space there is to work. After collection, I have the assistant hold off the vein for 1 to 3 minutes. Despite this, subcutaneous hemorrhage may cause some bruising and swelling at the venipuncture site.

For a complete blood count and plasma chemistries, I immediately make blood films followed by filling two hematocrit tubes. My next step is to place an adequate volume of blood into an appropriate anticoagulant tube, usually containing lithium heparin or sodium heparin. If serum is desired, such as for *Mycoplasma* serology, my next step is the serum separator tube.

My next favorite site is the subcarapacial vein, located beneath the nuchal scute or between the two midline cranial marginal scutes in tortoises that lack a nuchal scute. This works well in tortoises that are retracted in their shell if you have a long enough needle.

Lymph Contamination

There is an accessible vein on the lower aspect of the front limb where a large tendon dives into the back of the "wrist." This brachial vein is readily apparent in a large Galapagos and Aldabra tortoises that are stroked so they stand with legs extended. A smaller tortoise may sometimes be held in ventral recumbency with a front leg pulled forward and downward to access this vein. Although not as easy to find when a tortoise is in dorsal recumbency, this is an alternative method. Contamination with lymph is likely if the needle is moved around outside of the vein.

There are blood vessels on the dorsal and ventral aspect of the tail but I rarely use this due to likelihood of lymph contamination.

Cultures

Abscesses are common lesions noted on tortoises. If culture is desired, the skin should be aseptically prepared and a stab incision made. If the caseated abscess can be removed from the site, swab the outer layer of the abscess or the lining of the abscess capsule. Aerobic and anaerobic cultures are recommended.

Nasal flushes and tracheal flushes may yield appropriate material for culture. In my experience, culturing for *Mycoplasma* is often unproductive and does little to change my approach to a tortoise with minor nasal discharge. Tortoises with more severe respiratory diseases should have a tracheal wash submitted for culture. Sometimes it is necessary to drill a hole in the dorsal shell and use an endoscope to collect samples of lung tissue or exudate for culture.

Oral plaques and lesions are often the result of viral lesions but should still be evaluated for unusual bacterial or fungal specimens.

Cloacal cultures are often nondiagnostic except to confirm the presence of *Salmonella*.

Mycobacteria and fungi should always be suspected for granulomatous lesions that are widespread or are seemingly resistant to debridement and antibiotic therapy.

Feces

Fresh feces should be evaluated for parasites. I find direct fecal exams, with the feces diluted either with water or 0.9% saline, most productive. Although low levels of flagellated protozoa are normal for healthy tortoises, high numbers are of concern. *Hexamita* are always of concern given their propensity for invading the bladder and renal tissues and are readily distinguished from other flagellates by their rapid straight line travel across the field of view. Amoebas, particularly when coupled with abnormal amounts of RBCs and WBCs, are of concern. Pinworm ova are common in healthy tortoises, but other helminth parasites are of concern.

Feces that has been cooled or stored causes some protozoa to encyst and makes identification of significant parasites difficult. Some commensal ciliates encyst into structures that are commonly mistaken for trematode ova and I have seen many tortoises for second opinion that were being treated for "resistant flukes" that turned out to be ciliates.

Urine

If a tortoise urinates, I check the pH. Acidic pH suggests catabolic state from anorexia while an alkaline pH tends to suggest that the tortoise is still eating sufficiently.

Urine can also be assessed for casts, WBCs, and RBCs, as well as the presence of *Hexamita* and coccidia. Coccidia may be suggestive of a problematic disease, intranuclear coccidiosis.

Cytology

Fine-needle aspirates (FNAs) are often unrewarding because many masses are abscesses. Tumors may be productive but it is often difficult to make an interpretation other than an inflammatory mass or a suspected neoplasia unless there are extremely characteristic cells present. FNAs should be assessed with acid fast stain to rule out Mycobacteria as well as a more conventional Gram stain and cellular stains.

Lesions in the oropharynx should be lightly debrided and the underlying tissue sampled to have a chance of detecting herpesvirus or other inclusions. Touch preps should be made on any ulcerative lesions, including organs prolapsed from the cloaca. In addition to standard stains, cloacal touch preps should be assessed by wet mount since nematodes may often be discovered this way.

Tracheal washes should be evaluated with acid-fast stain, gram-negative, and cellular stains.

Biopsy

Biopsies are more likely to be diagnostic than cytological specimens simply because histopathology is better documented.

Histopathology requires standard preservation of tissues in 10% buffered formalin. For shell lesions it is important to include underlying bone as well as adjacent apparently healthy tissue. Often the biopsy specimens must be collected via surgical endoscopy of the lungs,

coelomic cavity, upper gastrointestinal tract, and cloaca, bladder, or proctodeum. Additional specimens should always be collected for culture and potentially samples for electron microscopy may be important. Any lesions noted during endoscopy should be photographed or videotaped in the event that a consultation with a referral veterinarian is desired.

External Parasites

Ticks, leeches, bot fly larva, and other ectoparasites are occasionally seen on chelonian patients. They may be preserved for identification. Restrictions on importation and interstate transport of some tortoises were enacted as the result of identifying ticks that were reservoirs for important foreign animal diseases.

Photographs

This is one of the most overlooked diagnostic tools in clinical practice. I highly recommend that a clinic have available a nice quality digital camera with high resolution and the capability for macrofocus. A ring flash is helpful for better illumination of some lesions. Digital photos can be readily enlarged allowing you to detect subtle lesions, such as petechia or septic blush that may not be readily visible with the naked eye. Photographing a patient on presentation and at recheck examinations will allow you to more objectively assess progress as well as giving a more comprehensive case to review if you consult another veterinarian.