THE REPTILE NECROPSY: COLLECTION AND SUBMISSION OF PATHOLOGIC SAMPLES

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HOW ARE TISSUES COLLECTED AND SUBMITTED FOR PATHOLOGY?
The most conventional method of submitting a biopsy is to place the biopsy in 10% neutral buffered formalin at approximately 10 parts formalin to 1 part biopsy, immediately after the biopsy is collected. This procedure insures adequate tissue perfusion by the formalin, and limits autolytic artifact (decomposition). Necropsies are pre-arranged and submitted on ice as quickly as possible. This prevents excessive decomposition of the carcass. To save on costs and to optimize tissue preservation, most veterinarians perform their own necropsies, collect the tissues into formalin, and submit them for histopathology.

HOW IS A NECROPSY PERFORMED ON A REPTILE?
The complexity and gauge of equipment is dependant on the size of the animal. Most equipment can be purchased at the local hardware store, but some equipment, such as dissecting scissors, forceps and T bars (for chelonians) may be purchased from medical suppliers. Generally, all that is needed are some fairly stout scissors, possibly a scalpel blade, pruning shears, a hacksaw and/or a striker saw (for chelonians). Sterile culturettes or sterile culture plates may be needed. Ideally, the instruments should be sterile, especially those used to collect tissues for culture, but this is not always practical in field situations. Equipment can be sterilized by dipping the tip in alcohol and then burning off the alcohol with a Bunsen burner or lighter.

Many reptile specimens are small, which makes necropsy a very easy procedure. For those animals less than 10 cm in length, incision of the coelomic cavity from the thoracic inlet to the pelvis will provide enough formalin perfusion and tissue fixation. The carcass can be placed whole in a container of formalin, at approximately 10 parts formalin to 1 part tissue. It is recommended that any desired visceral (intracoelomic) bacterial or viral cultures be obtained at the time the carcass is incised, prior to placing it in formalin. Imprints of tissues made on glass slides for cytologic evaluation should also be done at this time. Once placed in formalin, the tissues can no longer be cultured or used for cytologic imprints. For those specimens too big to be placed whole in formalin, the following procedures are recommended for the various tissues:

Skin:
Skin conditions are extremely common in reptiles, especially trauma, burns and infectious conditions. Survey the skin closely. Use a magnifying glass if necessary. Any abnormalities or asymmetrical discoloration should be noted and collected (and possibly cultured). The distribution and size of the abnormality should be succinctly noted in the history that accompanies the necropsy tissues, i.e. “Random on the back and legs”, “confined to ventral aspects of body (or legs)”, “tip of nose (or tail)”, etc. For chelonians, this information also applies to the carapace, plastron and bridges.

Opening the body wall:
Place the animal in dorsal recumbency (on its back). For snakes, reptiles and amphibians, use scissors to cut through the skin and coelomic wall from the tip of the mandibular junction to the cloaca. Gently part the skin and body wall with fingers, forceps or scissors and look for obvious abnormalities (lesions). Make a note of visceral arrangement and for apparent displacement of tissues. Take cultures of the lung, liver or kidneys with sterile instruments or swabs, before touching anything with your fingers. After collecting cultures, remove “the pluck”. (See below for opening a chelonian and obtaining the pluck.) This is done by cutting the tongue away from its attachments and pulling it through the ventral intermandibular space. Use a scissors or fingers to carefully dissect soft tissue attachments from the ventral aspect of the dorsal body wall. Apply caudal (backwards) traction to the tongue and dissect away the trachea, esophagus, lung etc all the way back to the cloaca. You now have all the viscera attached and can identify and inspect the tissues sequentially, collecting samples for histopathology and/or freezing (for future reference) as you go. This procedure may be difficult on large crocodilians, and the tissue collection and inspection may need to be done “in situ” (while still in the carcass). Also, some prosectors prefer to incise around the perimeters of the ventral body wall on large crocodilians (using pruning shears to cut through the ribs), rather than on the ventral midline, for better exposure.

Shell removal in chelonians:
There are several ways to remove the shell. In young chelonians or those with significant loss of shell bone, the shells can be separated at the bridge using a stout scissors. For larger animals it may be necessary to separate the shells using a striker saw or hacksaw. In all cases, the turtle is placed on its back, then tilted to a 45 degree angle. This angle allows gravity to shift the viscera downward, so they are not inadvertently incised during the sawing procedure. This is especially important for obtaining sterile cultures of the viscera and for avoiding a full urinary bladder. Take care not to go too deeply with the saw, cut the shell and no underlying soft tissues. Saw through both bridges in this manner. Then with the animal still in dorsal recumbency (on its back) use a scissors to cut through the skin at the attachment of the plastron (bottom shell), both front and rear. Use your fingers to carefully detach the soft tissue attachments from the top of the plastron, moving rear to front. If done correctly, the coelomic wall should be intact after removal of the plastron. For cultures, a small incision can be made in the coelomic wall to allow access to viscera. You will have access to liver, but not lung. After cultures are collected, use your scissors to cut the skin at the junction of the carapace (turtle still in dorsal recumbency), front and rear. Using a thick screw driver, wedge or T-bar, disarticulate the tail vertebrae and the cervical (neck) vertebrae from their attachments to the ventral aspect of the carapace. Carefully turn the turtle over and allow gravity to pull down on the viscera. Using your fingers, dissect the tissues away from the inside aspect of the carapace, starting from the back and moving forward. You may need to cut through the large skeletal muscle bundles in the shoulder region with a scissors. Let the carcass lay belly down on the necropsy table. If done correctly, you now have sterile access to the lung fields, and can culture the lung by making an incision through the wall and swabbing the inner surfaces. Obviously, you have to modify this technique to the best of your ability.
with the giant land tortoises and very large sea turtles that are too heavy to hold up for dissecting the carcass away from the carapace. After the shells have been removed (or just the plastron in case of large chelonians) and cultures have been collected, proceed with the chelonian necropsy as for other reptiles.

Endocrine tissues

The endocrine glands include the pituitary, pineal, thyroid, parathyroid, ultimobranchial gland, Carotid and aortic bodies, pancreas, and adrenal. By submitting the entire head, you automatically submit the pituitary and pineal. Disease processes involving these two glands are rare in reptiles.

The thyroid is important to examine for evidence of goiter or neoplasia. In reptiles, the thyroid is a single structure (not paired, as in mammals and birds), and located at the bifurcation of the great vessels. It is amber and appears multiloculated on cut surface. It is frequently mistaken for a mass or some form of disease process. Also in the region of the thyroid are small lobulated structures that represent the thymus, parathyroid and ultimobranchial bodies. These microscopic glands are located along the greater vessels and at the base of the heart, and finding them is very difficult. They are usually detected fortuitously in microscopic sections of the greater vessels and heart base.

The pancreas has both exocrine (digestive) and endocrine functions. Examination of the pancreas is important, because it develops microscopic changes indicative of nutritional status. Some infectious disease processes have a tropism for the pancreas, such as paramyxovirus and monocercomoniasis in snakes. Among reptiles there is considerable variation in the location of pancreas. In many snakes and some turtles, it is fused with the spleen (splenopancreas) and located in the mesentery next to the gut and gall bladder. In some species, the pancreas is an isolated gland in the mesentery adjacent to the duodenum (small intestine where it exits the stomach). Some species have both isolated pancreas and splenopancreas.

The adrenal gland is an important tissue to examine microscopically because it develops microscopic changes that may indicate stress, which can be difficult to evaluate in an otherwise stoic reptile. The adrenal is paired in reptiles. In reptiles, there is considerable variation in shape and some minor variation in location of the adrenal. Generally speaking, it is a tubular or ovoid structure in the mesovarium or mesorchium (thin membranes attached to reproductive tract) on the medial aspect of the gonad. In snakes, it is especially long, and can be anterior or posterior to the gonad, so careful prostate is required to be sure both gonad and adrenal are identified prior to collection of each.

Reproductive Tract

Common reproductive problems in reptiles include bacterial infections, neoplasia, pre- and post ovulatory follicular stasis, and prolapse of the hemipene, uterus or cloaca. Routine collection of these tissues is very important. Gonads are paired. Females have a paired oviduct (uterus) and males have a paired epididymis and vas deferens. Male chelonians and crocodilians have a penis. Lizards and snakes have hemipenes located caudal to the cloaca on both sides of the ventral aspect of the tail.

Urinary Tract

Common disease conditions in reptiles include renal gout, urolithiasis (stones), nephrocalcinosis, bacterial and parasitic infections, and neoplasia. The kidneys are paired, and generally located in the caudal coelomic cavity. They are especially caudal in the chelonians and some species of lizards (iguanas) and are frequently missed at necropsy. The kidneys are ovoid in most species of reptiles. In snakes they are lobulated and cylindrical. Commonly, the gonads are mistaken for kidney. It is important therefore to identify “in-situ” the kidneys, gonad and adrenal prior to collecting these tissues. Chelonians and some lizards have a urinary bladder. Snakes, crocodilians and some lizards do not.

Alimentary Tract (Mouth to cloaca, including the liver and pancreas)

Disease conditions of the alimentary tract are especially common in reptiles and include a number of bacterial, fungal, viral and parasitic infectious diseases, foreign body obstruction, intussusception, stasis (loss of gut contractions), and rectal/cloacal prolapse. Hepatic diseases include infectious processes, neoplasia, toxicosis, lipidosis and melanosis. Representations of all portions of the gut, including the oral cavity (included with the head), esophagus, colon, rectum and cloaca.

Hematopoietic and Lymphoid tissues (spleen, thymus, bone marrow, peripheral blood)

Examination of these tissues provides clues as to immune status, anemia, and various infectious and nutritional problems. Blood films (on slides) can be made from heart blood at necropsy if the blood is not clotted. The spleen is generally a small ovoid brown/red or tan structure located in the mesentery attached to the small intestine. It can be difficult to find but is important to examine for the presence of infectious disease processes. Evaluation of overall lymphoid cellularity can give a crude estimation of immune status and can also provide evidence of exposure to stress. The thymus is a lobulated structure in the fascia close to the thyroid (see discussion of thyroid), and is responsible for production of lymphocytes. Bone marrow of the long bones is gelatinous in lizards and crocodilians but “trabeucular” and difficult to collect separately in snakes and chelonians. It is generally examined as part of the microscopic bone exam.

Cardiovascular system

A broad array of disease processes affects the cardiovascular system, such as atherosclerosis, bacterial endocarditis, degenerative cardiomyopathy, and mineralization of the greater vessels. Submission of the entire heart and a short length of cardiac outflow vessels (greater vessels) in formalin is recommended. Opening the heart for formalin perfusion is helpful, and may reveal lesions worthy of culture prior to formalin submersion.

Respiratory tract

Numerous disease processes affect the respiratory tract, especially infectious diseases and mineralization due to metabolic or nutritional diseases. Submission of the entire head covers the nasal cavity and larynx. Representative sections of the trachea and lung should always be submitted. In reptiles the lung is a hollow sac with a honeycomb appearance on the inner surface. The anterior portion has a thick wall and has respiratory function. The posterior portion is thin-walled and resembles an avian air sac. The anterior portion is generally more useful for diagnostic purposes, but
submission of both regions is recommended. Some snakes have only one well-developed right lung, and one rudimentary left lung or no left lung.

**Musculo-skeletal system**

Examination of this system is very important, as it provides useful information regarding current and past nutritional status. There are also a number of traumatic and infectious conditions that affect the musculo-skeletal system. When whole carcasses are submitted, the musculo-skeletal system is covered. For carcasses too large to submit whole, an effort should be made to submit the head, a segment of vertebral column and at least one long bone with articular surface, such as the femur, tibia, humerus or radius.

**Nervous system.**

Diseases of the nervous system are occasionally seen in reptiles, and are usually manifestations of other visceral disorders such as gout, neoplasia, intoxication or infectious disease. The brain and cord are examined when the entire head or vertebrae are submitted. For those animals too large for submission of an entire vertebra or head, prosection of the spinal cord and brain will be required. The mouth should be taped shut so fingers are not impaled on teeth or fangs, and the top of the skull should be removed with a striker saw or bone rongeurs for exposure to the brain. The top of the vertebral body (dorsal process) can be removed in a similar pattern for access to the spinal cord.