Necropsy Review and Diagnostic Sampling

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Abstract

While many practitioners recognize the utility of necropsy, some are hesitant to perform necropsies. The ability to perform a thorough necropsy and submit appropriate specimens for laboratory analysis is essential to obtaining a diagnosis and maintaining herd health and is a valuable service for clients. While submission of the entire body to a diagnostic facility for necropsy by a diagnostic pathologist may be ideal, field necropsy may be more practical due to many factors including cost or difficulty of shipping, postmortem interval and autolysis, distance to the nearest diagnostic facility, and cost of the necropsy. Necropsy may be particularly informative if morbidity and mortality exceeds what is expected, with suspected treatment failure, if clinical signs are unusual, or in cases of sudden death. Necropsy is a time and labor-intensive process, however, the ability to perform this type of examination and collect the appropriate samples can be vital to obtaining a definitive diagnosis.

Keywords: necropsy, food animal, diagnostic sampling

Preparation

Case selection, necessary tools, and location are important considerations in the preparation stage. In some cases, case selection is obvious,but in some cases it may not be so simple. With infectious disease, chronically affected animals may yield the most obvious lesions, but the inciting organism may no longer be present and there may be opportunistic infections that muddy the waters. If an animal has been dead for some time before it is found, it may be better to euthanize a sick animal and immediately perform a necropsy to obtain diagnostic-quality samples. On the other hand, it may not be possible to convince the owner to allow euthanasia of additional animals, and some information may be obtained even from necropsy of a slightly autolyzed animal.

Tools of the Trade

In general, the tools that are required for a complete necropsy are: personal protective equipment (coveralls, boots, goggles, gloves) a sharp knife, a cleaver or hatchet, a handsaw, heavy duty brush trimmers, scissors, forceps, and scalpel. It is ideal if there are two sets of the smaller instruments to enable collection of relatively uncontaminated samples for testing such as bacterial culture. A good sharp knife is the most important instrument used in a necropsy.[1] In the authors' experience, injuries are more likely to occur with blunt instruments as you are less likely to saw at the tissue and cause injury from instruments slipping.

Additional equipment and supplies required will depend on the samples to be collected and shipping/preservation needs. In general, the supplies needed include a cooler and ice packs for fresh tissues, 10% neutral buffered formalin, clean whirl-pak bags for fresh tissues, and red top

tubes for bodily fluids. In some cases, other collection tubes may be required. Water and soap/disinfectant will also be needed for cleanup.

Choosing a location for the necropsy is another important factor. Sometimes an ideal location cannot be chosen due to where the animal died and lack of ability to move it, but if location can be chosen, it is best to choose an area from which other animals can be excluded. Additional factors to consider are: ability to disinfect, equipment access (truck and trailer, etc.), availability of water for clean-up, safety and comfort, and convenience.

External Examination and Initial Approach

The process for the initial approach to a necropsy is covered in many texts and articles, two of which have been chosen as reference material.[1, 2] The reader is referred to these publications for greater detail than is possible in these proceedings. By convention at the Alabama State Diagnostic Laboratory animals are placed in left lateral recumbency (in right lateral recumbency the rumen blocks access to many of the major abdominal organs). Because this is the procedure we use, it is the basis of the following description, but it is more important that the necropsy is performed the same way every time than that any specific protocol is followed.

The first physical step in the necropsy procedure is external examination. External examination should be similar to that on a live animal (temperature, pulse, respiration obviously excepted). Sampling of certain bodily fluids (vitreous and aqueous fluid, blood) are also done at this point and ocular fluid can be checked for nitrate with commercially available test strips. Examination of the musculoskeletal system can be done partially with the initial dissection, and if specific joints or areas have pathology, these may be examined by opening joints as needed. Begin the necropsy by stabbing into the right axilla; cut through the skin from the interior to the exterior to

avoid dulling the knife on hair. Cut the muscles connecting the scapula to the thorax and reflect the limb back over the dorsum (Figure 1A, B). Reflect the hind limb similarly by cutting through the skin and medial thigh muscles and the coxofemoral joint to reflect the hind limb (Figure 1C, D). Make a ventral midline incision connecting these incisions and reflect the skin to the level of the spine (Figure 1 E). Extend the skin incision up to the mandibular symphysis and reflect the skin away from the ventral neck. Examine the subcutis and musculature.

Open the abdomen by making a long cut just caudal to the ribs (Figure 1F). Once the opening is large enough, holding the back of the blade between your first finger and thumb, with the handle in the palm and pointing down toward the wrist, push into the incision with the butt of the handle first. Holding the knife as described (handle down into the abdomen, point of the knife facing out and up), cut the abdominal wall away dorsally along the ribcage and caudally toward the pelvis, creating a flap that will be pulled ventrally to expose the abdominal organs.

At this point, the thoracic organs are still covered by the ribcage and the diaphragm. To expose the thoracic organs, pull the intestines caudally and ventrally to access the diaphragm. Check for negative pressure by stabbing into the diaphragm. It should become flaccid while air rushes into the thoracic cavity. Cut the diaphragm away near its attachment to the ribs. Cut a line in the thoracic musculature over the ribs that extends from the last rib, along the dorsal aspect of the ribs to the dorsal thoracic inlet. Using the brush/hedge trimmers, cut the ribs along the line incised in the muscles (Figure 2A, B). If hedge trimmers are not available, an axe may be used to fracture the ribs by working cranially beginning at the last rib. After the first few ribs, it may be helpful to cut a hole in the intercostal muscles to provide a handhold for an assistant. If you wish to leave the ribs attached to the body for easier clean up, cut along the inside of the ribs at the costochondral junction. The ribs can then be pulled ventrally, fracturing along the line at the

costochondral junction, and remain attached to the body. Alternatively, the ribs may be removed from the body by cutting along the costochondral junction with the hedge trimmers (Figure 2C).

Examination of Thoracic Organs

Examine the abdominal and thoracic organs for position, color and other obvious abnormalities before removing or sampling. It is best to sample tissues for microbiological testing before too much manipulation (and contamination) and organs such as lung, liver, or kidney before the gastrointestinal tract to prevent contamination. Use clean or sterile instruments to collect these specimens. Open the pericardial sac and note any fluid or fibrin, etc. in the pericardial sac.

Thoracic organs may be left with some attachment to the body to facilitate clean up or may be removed from the body entirely. Free the tongue from the mandible by cutting along the medial aspects of the mandible. Pull the tongue ventrally and examine the oral cavity and larynx. Continue to pull ventrally on the tongue, incise along the soft palate, and pull down until you reach the pharynx. Cut between the bones of the hyoid at either side of the pharynx. Continue to pull down and cut behind the trachea and esophagus until they are freed to the level of the thoracic inlet

To remove the thoracic contents, continue to pull the trachea and esophagus caudally and expose the cranial mediastinal tissue, then begin cutting through this tissue. As you cut, the lungs, heart, and thymus (if present) will come away from the thoracic wall, allowing examination of the cavity. If you are removing the organs for examination, continue cutting until you reach the diaphragm, and cut all attachments between the thoracic organs and the ventral thoracic cavity. To remove the thoracic organs, three structures must be cut: the aorta, the esophagus, and the caudal vena cava. Remove the thoracic organs, trachea, esophagus, and tongue (the pluck) from

the body. If you are leaving some attachments to the body, cut the attachments dorsally and cut through the aorta, but leave the ventral tissues, esophagus and caudal vena cava to anchor the pluck to the body. If the ribs cannot be cut or a more cosmetic look is desired (usually at client request) the thoracic organs to the level of the distal trachea may be removed by cutting through the diaphragm, reaching into the thoracic cavity, grasping the trachea, and while pulling caudally, cutting through the trachea. The trachea can then be used as a hand-hold to pull the thoracic organs caudally while cutting the attachments as previously described.

Make several cuts across the tongue and examine it. Using a knife or scissors, open the esophagus from the larynx to the diaphragm. Examine the esophagus for bloat line, which will appear as a relatively sharp demarcation between pale (cervical) and congested (thoracic) areas due to increased intra-abdominal and thoracic pressure from bloating of the rumen. Once the esophagus has been examined it can be cut away to facilitate examination of the trachea. Open the trachea along the dorsal ligament from the cranial aspect to the tracheal bifurcation of the lungs. Extend the incision into the pulmonary tissue along the mainstem bronchi. Examine the trachea for mucosal pathology, exudate, fluid, or foreign material. Locate the main airway into each lung lobe and open it as far as you are able. Running with the airway (ventral and dorsal) are the pulmonary arteries and veins. These should also be opened and examined.

Examination of the heart is done while still attached the lungs to preserve orientation. Find the pulmonary artery (the auricles point to it). Cut down from the pulmonary artery near the right ventricle's junction with the interventricular septum through the right atrium and ventricle toward the apex of the heart. Then turn and cut up along the other side creating a V-shaped flap from the right atrium and ventricle. On the left side, cut through the center of the left free wall to

the apex of the heart, exposing the mitral valve. After the mitral valve has been examined, cut through it to reveal the aortic valve and aorta.

Examination of Abdominal and Pelvic Organs

To prevent contamination, the parenchymal abdominal and pelvic organs should be examined and samples taken before the gastrointestinal (GI) tract is opened. The right kidney is usually embedded in fat but may be visible in thin individuals. Bluntly dissect the kidney from the fat and cut the renal vein and artery. Cut the kidney in half longitudinally, peel off the capsule and take a cross section through a lobule, including cortex and medulla. The adrenal gland is in the perirenal fat, medial and cranial to the kidney. The left kidney is located by flipping the intestines dorsally out of the body cavity. Open the bladder to examine the mucosal surface. If it is necessary to examine the pelvic organs intact, based on the history and clinical signs, the pelvis may be opened to facilitate examination of the distal genitourinary tract. A saw is used to cut the pelvis through the pubis and ischium at the obturator foramen and through the ilium a few centimeters cranial to the acetabulum. This section of the pelvis can then be removed to access the pelvic organs.

The liver, gall bladder, and pancreas are examined next. Examine the liver for size, color, shape, and texture, then make several serial slices through the organ (bread loaf). Find the entrance of the bile duct into the duodenum. Place your fingers here and squeeze the gall bladder to assess patency. Open the gall bladder to examine the mucosa.

Generally, spot checks of the various intestinal segments are sufficient. When opening segments of intestine, cut near the mesenteric aspect to preserve the Peyer's patches. Open the abomasum along the lesser curvature (including extension of the incision into the pylorus) and examine the

content as well as the mucosa. When examining the content, it is sometimes helpful to dilute it with water and spread it across an even colored surface. Abomasal parasites such as Haemonchus spp are very small and easy to miss. Open the reticulum and omasum. Open the rumen by cutting through the dorsal sac and removing the content to allow visualization of the mucosa. Rumen pH may be tested using commercially available test strips before the content is removed. The spleen will be on the down side of the rumen and is easiest to access once the rumen is empty.

Examination of Central Nervous System

Removal and examination of the brain is often omitted from field necropsies,[2] but valuable information may be lost if examination of this organ is neglected. The head is removed by pushing the head back and cutting through the atlanto-occipital joint ventrally and continuing the cut through the spinal cord, nuchal ligament and musculature. Remove the skin and muscle from the skull. Using an axe or cleaver, cut through the frontal bone. Connecting cuts should be made from this initial cut across the frontal bone, along the sides of the skull and connecting through the foramen magnum. This portion of the skull can then be lifted away to reveal the meninges and brain. The dura mater will have to be cut before the brain can be removed. To remove the brain, transect the cranial nerves. This can usually be done by blunt dissection and is easiest if the head is tilted back onto the condyles to allow gravity to pull the brain away from the floor of the skull. The pituitary and trigeminal nerves can then be examined. The brain may also be removed using a hand saw if it can be adequately stabilized, or the entire skull may be cut in half longitudinally and each half of the brain removed separately. If testing for bovine spongiform encephalopathy is needed and is the only testing to be performed on the brain, alternate methods are available to remove only the cerebellum and brain stem.[2]

Sample Collection

Collecting the right samples in the appropriate manner and shipping method are important to obtaining a correct diagnosis. When in doubt, call the laboratory to which you are submitting the samples. In general, samples submitted to a diagnostic laboratory should include fresh and fixed tissue from all parenchymal organs, all gross lesions, and any organs that you suspect are the source of disease. Depending on the disease process suspected, additional samples may be required, including, but not limited to: serum, ear notch for BVD testing, placenta (especially on abortions), rumen content, and feces. If you are unsure of which samples to collect, consult the laboratory website for submission instructions or call the laboratory directly for instruction.

While the samples in formalin should be relatively small to allow fixation (less than 1 cm thick) and the volume of formalin should be large (10:1 formalin to tissue), err on the side of collecting more tissues. At the time of necropsy, it is better to collect more than you think necessary. It is better to have samples and not need them than to need them and not have them, and once the necropsy is complete, it is rarely possible to return to the carcass for more samples. Fresh tissues should be individually bagged and labeled, including the organ and animal identification. If you have questions about collection, transport media, or shipping methods, call the laboratory and they will direct you. Samples should be placed in a primary receptacle (whirl-pak bag, screw top tube, etc.), then secondary leak proof containers before being placed in the shipping box. This prevents leakage of potentially hazardous materials. Fragile containers, such as blood collection tubes, should be individually wrapped so that they do not contact each other (to prevent impact and breakage).The U.S. Department of Transportation has published guidance documents for shipping of potentially infectious materials.[3]

Filling out the submission form completely, including detailed history, will allow the diagnostic laboratory to make some decisions about additional testing should it be required or if you do not know which testing is appropriate for the case. The importance of a detailed history cannot be overstated. Photographs of lesions are also helpful if included.

Discussion

This is an abbreviated description of necropsy techniques as all potential variations and sample collection procedures cannot be detailed within the limits of this manuscript. This document and the references included should enable the practitioner to perform necropsies and develop facility and confidence in their ability to perform the procedure. Specific questions about sample collection and shipping should be directed to the laboratory that samples will be submitted to. Diagnostic laboratories vary in the array of tests offered, and the tests available will change from time to time as new technologies are adopted. When in doubt, contact the laboratory or the pathologist to whom you will direct samples, this will enable the best results from your efforts and provide accurate, timely diagnostics to your clients for management of health concerns within their herds.

References

1. King JM, Roth-Johnson L, Dodd DC, Newsom ME. The Necropsy Book: A guide for veterinary students, residents, clinicians, pathologists, and biological researchers: The Internet-First University Press; 2014.
2. Mason GL, Madden DJ. Performing the field necropsy examination. Vet Clin North Am Food Anim Pract. 2007;23(3):503-26, vi.
3. Department of Transportation. Guide to Changes, Effective October 1, 2006. Transporting Infectious Substances Safely 2006 [Available from: https://hazmatonline.phmsa.dot.gov/services/publication_documents/Transporting%20Infectious%20Substances%20Safely.pdf.