

Techniques in Necropsy: Pathway to Knowing Everything

Title Page

Good afternoon everyone. My name is Chris Koivisto and I am an upcoming second year student here. I would like to begin by thanking Doctors Stoskopf and Kennedy-Stoskopf for putting together this summer series and for my classmates, Sam and Jenessa for inviting me to speak here today about something that I am very passionate about and am more than happy to share with you.

There is a joke in the world of veterinary medicine- Pathologists know everything; only it's too late.

The post-mortem exam

A true post-mortem examination will involve any or all of these listed components. A detailed clinical history of the patient will be essential when it comes time to interpret any data collected during the post. Histopathology is the microscopic examination of tissues. It essentially gives a name to the observed gross changes and often may establish an etiology. Bacteria and viruses may be isolated from tissue or body fluids. Tissue, such as liver, may be sampled and analyzed for toxins. Molecular diagnostic tests, such as PCR have become increasingly popular and may be used to identify the presence of an infectious agent or genetic alteration. All of these procedures begin with a necropsy

Definition

A necropsy, in my opinion, is the most valuable diagnostic tool among a veterinarian's arsenal. Zoos are probably most consistent in incorporating necropsies as part of their standard health program. Many years ago, I spent a day shadowing a veterinarian from the Toledo Zoo in Ohio. Every animal that dies is necropsied; I recall watching him post a finch that had been found dead overnight in an exhibit.

Unfortunately, it is overwhelmingly under-utilized in small animal private practice. How many of you have worked in a small animal clinic? How many necropsies were conducted? In the three practices I have worked in, probably less than five animals per year receive a post-mortem exam.

Why do a necropsy?

A necropsy may be conducted to gain diagnostic evidence of unknown or suspected antemortem disease. A mass seen on radiographs or ultrasound or a beloved pet found dead in the owners fenced-in backyard.

Necropsy may be used for preventative medicine, especially in regards to any herd health management program. Increased mortality or morbidity among a population of animals calls for an investigation into the cause. Indeed, sometimes it may be necessary to examine a clinically healthy animal to find the answer.

Probably the most important benefit of necropsy, especially for our profession, is the opportunity to further educate ourselves. By trying to fully appreciate and understand the changes that have occurred during a patient's illness and comparing the gross pathology

with the clinical signs, we will be more likely to recognize that disease in the future and obtain a better prognosis.

Necropsy Facility

It is ideal to have an area dedicated solely to necropsies away from animal housing. This will prevent possible spread of disease to healthy individuals. The room should be well ventilated and surfaces should be easy to disinfect. Stainless steel work surfaces are great and some tables have a downward draft ventilation system to help protect the prosector from aerosolized particles. Biosafety hoods may be useful when posting small animals as well. Hydraulic tables are wonderful for large animals.

If doing necropsies in the field, isolate yourself from the healthy animals and be sure to properly dispose of the remains and disinfect the area as much as possible.

Basic Equipment

Most necropsies do not require state-of-the-art technology to get the job done. Your best friend is a sharp knife. Contrary to what I once believed, the steel does not sharpen the blade, but it does even out the bevel which will keep it functional. You will need some sort of tool to cut through ribs. Tree pruners tend to work well. A handsaw works best for thicker bones. Rongeurs are used to remove the calvarium and dorsal regions of the vertebrae to access the brain and spinal cord. A metric ruler to measure lesions and appropriate containers to hold tissue.

Goals of necropsy

The primary goal of necropsy is data collection. The specifics of collecting data vary depending upon what questions are to be answered afterwards. Such questions are usually part of the clinical history and should be reviewed before starting.

It is also important to not let the questions asked by clinicians bias your overall approach towards dissection. A prosector must be systematic in their technique; otherwise the likelihood of overlooking important information increases significantly. A good method to prevent the likelihood of lost information is by creating a necropsy record.

A necropsy record should contain the animal's identification (name, eartag, tattoo, microchip, etc), species, breed, sex and age. The date and or time of death, the method of euthanasia, and the date and time of necropsy should be noted. This is important as certain gross changes may often be contributed to euthanasia methods and normal autolytic changes from too much time passing between time of death and the post exam.

A list of routine tissues to be examined is very useful and may be checked off as the exam progresses.

And lastly, space to describe gross lesions. A good description should paint a picture of the lesion in the mind of the person reading it. They should be descriptive but not interpretive. Interpretation occurs only after all the data has been collected.

Lesion descriptions should include:

- Location (name of tissue, specific location on tissue-right lobe, cranial, etc. Draw picture if desired)
- Color (keep it simple, use primary colors when possible, don't describe a box of crayola crayons)
- Size (metric units, give an actual number, not an estimate-“baseball-sized”)
- Shape (dimensional)
- Consistency and Texture (touch it. Soft vs. firm; friable, granular, etc)
- Number/Extent (give an actual count; if diffuse-indicate percentage of organ affected)
- Odor (okay to compare to known smells)
- Surface appearance (ulcerated, hairy, smooth, glistening, dull, etc)

Kidney picture

Bovine, left kidney, capsule, single mass, white, 4cm diameter x 2cm thick, firm, smooth

Tissue sampling

Methods of preservation for samples taken during necropsy may limit which types of diagnostic tests will be capable of yielding useful information.

Cytology

Samples for cytology should be prepared before tissue is exposed to any sort of fixative. Touch impressions are made from the pressing the cut surface of a tissue against a clean slide. It is important to blot away excess blood with a paper towel prior to making the impression.

Fine needle aspirations are made by inserting a needle and syringe into a solid mass and pulling back onto the plunger several times in an effort to draw up cells from the interior, then expelling them onto a slide. It should be noted that large masses often have necrotic center cores and samples taken from those areas will not be diagnostic.

Fluid and exudates may be collected with a cotton swab and rolled onto a slide.

Skin lesions may be scraped with a scalpel blade and the collected material spread out onto a glass slide.

Light microscopy

Histomorphological review under light microscopy is the most common diagnostic test performed from samples collected during necropsy. During necropsy, representative tissue samples are collected and placed in 10% neutral buffered formalin for fixation. The minimum ratio of fixative to tissue is 10:1. Formalin is only capable of penetrating about 0.5mm of tissue in a 24-hour period. Therefore most samples should not exceed 1.0cm in thickness or the center will be too far decayed for interpretation. A few exceptions include brain and testes, which are too delicate to be sectioned thin prior to fixation and should therefore be fixed whole.

When sectioning lesions, it is preferable to take samples that include the junction between grossly appearing normal and abnormal areas. Also collecting samples with scissors will introduce crushing artifacts; using a sharp knife or scalpel blade is best.

Histochemistry involves taking advantage of the differing chemical properties between various cell components and using specific dyes to highlight those components. Examples include gram, acid fast, and silver stains to highlight infectious organisms. Immunohistochemistry utilizes enzyme-labeled antibodies capable of binding specific proteins within either the nucleus, cytoplasm or plasma membrane. An example would include proinsulin granules found only in beta cells of the pancreas.

Microbial Culture

Collection of samples for microbial isolation must be done so using aseptic techniques to avoid contamination. Ideally, these should be the first samples that you collect. Sterile scalpels and swabs are convenient. Instruments may be disinfected by soaking in alcohol and then passing the instruments through a flame.

Vials containing growth media specific for certain bacteria, fungi and viruses are available from a reference laboratory. Areas to swab would include pus, abscesses and GI contents. Whole pieces of tissue may be collected and placed in sterile containers, such as Whirlpaks. If sampling pieces of tissue, be sure to include areas near the lesion's edges as this will increase your likelihood of getting organisms. Tissue taken for virus isolation should be frozen at -20 Celcius and may be preserved long-term at -80 Celsius.

Ectoparasites may be preserved in alcohol.

Care should always be taken when necropsying an animal suspected of dying from an infectious disease. Gloves, and clothing, such as coveralls and rubber boots which can be easily disinfected should be always be worn. If conditions or equipment are likely to allow aerosolization of tissue, then masks, goggles and/or face shields should be considered to minimize your risks of exposure.

Molecular analysis

Molecular diagnostic techniques such as PCR are increasingly used to identify the presence of pathogens. Formalin tends to degrade nucleic acids over time limiting this application. Frozen tissues yield better results especially when flash frozen in liquid nitrogen and stored below -70 Celcius.

Toxicology

Tissues collected for toxicologic analysis are usually submitted either fresh or frozen. Some compounds may require special handling, such as being wrapped in aluminum foil, so it is best to consult the laboratory prior to necropsy. Tissues commonly sampled include liver, kidney, skeletal muscle and fat.

Electron Microscopy

1% Glutaraldehyde is the fixative of choice for samples destined to be examined by electron microscopy. To maximize results, it is suggested to perfuse the tissue with fixative. Perfusion is accomplished by injecting fixative through the vasculature of an organ. It is also often possible to use paraffin embedded, formalin fixed tissue.

General Necropsy Procedure.

All necropsies, regardless of species begin with a review of the history, obtaining a weight, and performing an external examination. Afterwards, the animal is properly positioned in preparation for the internal examination.

Proper positioning will vary between species but the overall goal is to enable an approach that allows visualization of the viscera in situ. This allows the prosector to identify any displaced organs or excessive fluid within the body cavities. Once complete, the prosector is free to begin systematically examining each organ.

All tissues examined should be removed and thoroughly palpated. Hollow organs should be incised and their mucosal surfaces examined. Tissues having a cortical and medullary regions, like kidney and lymph nodes are cut in half to examine both portions. Large, solid organs such as spleen and liver can be breadsliced to look for any lesions not evident from the surface.

Carnivores and ungulates are placed on their left lateral side. The right limbs are reflected dorsally. The skin is incised from the midline and reflected dorsally as well, while the right lateral abdominal wall is reflected ventrally. This should allow visualization of at least the liver and intestinal tract. At this point, any excess fluid in the abdomen may be collected and measured. The diaphragm is punctured while listening for the sound of air to rush into the thoracic cavity. The ribs are removed and the diaphragm incised to reveal the thoracic cavity.

To examine birds, first wet the feathers to help visualize the skin and decrease possible contamination of any cultures taken during the internal exam. Birds are placed on their backs and the skin is removed to expose the keel. Make an incision near the caudal portion of the keel so that you can lift up on the sternum while cutting along either side of the pectoral muscles. Reflect the pectorals and sternum cranially so that the viscera may be observed.

Reptiles are placed on their back and incised along the ventral midline. Those that are wider laterally rather than dorsally, like chameleons may be positioned similar to carnivores.

For turtles, the plastron is sawn away from the carapace and removed.