Necropsies (Post-Mortem Examinations)

The information obtained from post mortem examinations can assist in: determining treatment protocols of live animals and discovering disease processes that may be present in wild populations. Necropsy findings may be important evidence in criminal cases involving wildlife (poisonings, electrocutions, gunshots, oil spills, abuse, etc.).

GENERAL NECROPSY PROCEDURE

Set-up for necropsy

The room should be very well **ventilated**, at some distance from food preparation, surgery and areas for live animals, and have ample light and running water.

All personnel in this room should wear **masks and gloves**—there is always a risk of exposure to **zoonotic diseases**.

Limit the access to the necropsy area to trained personnel (to limit the exposure and spread of potential pathogens throughout the facility).

Label all collection containers and forms with the animal's case number, date and other pertinent information.

Be prepared to list/describe any photos taken during the procedure.

Gather supplies: scalpel or knife; scissors, forceps, hemostats, shears/rib-cutters.

Necropsy protocol

1. Complete external physical examination, beginning with a body weight (see history). Assess feather/fur/skin condition, bones for fractures, skin, mucous membranes, nutritional status, external parasites, etc. If possible, take at least one whole-body radiograph of the intact carcass (review any radiographs provided).

2. Incise the skin at the atlanto-occipital joint to reveal the foramen magnum. Incise over top of head from formen toward beak/nose (rostrum), and push skin to either side. Place shears in formen magnum and cut each side towards eyes, then back toward the rostrum. Gently lift off the top of the calvarium and examine brain and meninges. Examine eyes and ears.

3. Position the animal in dorsal recumbency. Very large mammals should be positioned in left lateral recumbency. With a sharp knife or scalpel blade, make a skin incision along the ventral midline, reflect the skin laterally, inspect the underlying subcutis and muscles. Make several cuts into the pectoral muscle of birds (quads and gluteus of mammals), looking for areas of discoloration, fibrosis, hemorrhage, etc.

4. Extend the skin incision cranially to the throat and caudally to the cloaca/rectum.

5. Note the interclavicular air sacs at the entrance to the thoracic cavity of birds. Note the conditions of these sacs.

6. Incise the abdominal muscle along the caudal border of the sternum, carefully to avoid contaminating the viscera.

7. Using your hands, force the legs apart or use scalpel to cut ligaments of the head of the femur (disarticulate the hip joints). This will help stabilize the carcass on the table. Do the same for the wings/forelimbs by cutting the ligaments at the head of the humeri.

8. Extend incisions through the linea to the cloaca/rectum, exposing the caudal viscera. Note the presence or absence of abdominal fat and the condition of the abdominal air sacs in birds and omentum in mammals. Observe the diaphragm (mammals) and examine the viscera *in situ*. Culture liver if indicated.

9. Incise the pectoral muscle along the lateral margins of the sternum. Using shears, cut through the ribs, and clavicles [in birds, disarticulate or cut the coracoids], and carefully lift the sternum and remove it. Note the condition of the heart and lungs, and the thoracic air sacs in birds. Collect heart blood for possible culture. Collect swab from lungs if indicated.

10. Examine gonads and adrenal glands and locate the spleen. [In birds, this is easiest to do by reflecting the abdominal organs to the bird's right, to expose the left kidney, adrenal, gonad and spleen]

11. Using shears, cut through the lateral commissures of the mouth. Note the condition of oral cavity. In birds, use shears to remove the upper beak just behind the nares; inspect the nasal cavity and infraorbital sinuses. Collect culture swab if indicated. In mammals examine the oral cavity and sinuses (sinus examination is difficult in most mammals and may require a ban saw or hack saw to accomplish).

12. Important: note colors and sizes and spatial relations of all viscera before anything is removed! Then remove *en bloc* the esophagus, trachea, lungs and heart, digestive organs. Or, remove the trachea, lungs and heart separately from the esophagus, proventriculus, gizzard (stomach) and remainder of the viscera.

-- Separate the trachea, syrinx/pharynx and lungs. Open and examine all cavities. Culture if necessary. Collect all parasites for examination.

-- Separate heart and great vessels. Open and examine.

-- Open esophagus, proventriculus, ventriculus (stomach) intestines, ceca, cloaca/rectum. Note color of serosa and mucosa, and contents, and collect any parasites for identification. If indicated, collect culture swab before handling.

-- Examine pancreas, gall bladder, liver, spleen.

13. Examine kidney and pelvic nerves and vessels, and the pectoral/brachial nerves.

14. Open the joints and collect culture swab if indicated.

15. Make impression smears (touch impressions) of any lesion on a clean glass slide.

16. Bag, label and properly dispose of any remaining portions of the carcass.

Sample/Evidence Collection

1. **Records:** It is very important to keep good necropsy records, if possible, by using a tape recording system or a person who can take notes dictated by the prosector. Use a standardized protocol and results sheet to facilitate reporting the results of individual necropsies and make the overall analysis easier in the future.

2. **Fixatives for histology**: Use 10% neutral buffered formalin to fix the tissues to be examined histologically. Avoid freezing tissues intended for histological examination. If electron microscope studies are desired, fixatives other than formalin are preferable; consult the laboratory that will be doing the study.

3. **Volume of formalin required**: It is very important to use a container large enough to hold your tissue specimens and enough formalin to fix them. There should be a ratio of 1 part tissue to 10 parts formalin for best results.

4. **Size of specimen to collect**: Formalin diffuses into the tissues, so sections too large will continue to autolyze even in formalin. The thickest dimensions should be <5mm.

5. **Tissues to save**: When possible, a full range of tissues should be saved—those with lesions and those that appear grossly normal. Those with obvious lesions can be submitted immediately for histology, and other tissues can be saved for examination at a later date. This is particularly helpful with wildlife cases, as the pathologist may not be familiar with each species!

6. **Virus isolation**: Generally, tissues should be harvested sterilely (as you would for bacteriology). They should be placed in a sterile container, in special medium or frozen (-70 degrees C). Consult the laboratory to be used to be sure the necessary materials are on hand, and to know how to package the samples for shipment to the lab

7. **Toxicology testing**: This is especially important in attempts to prove intentional intoxication as the cause of death. Chemically clean glassware, Teflon or other appropriate containers must be used when testing for certain toxins. Many protocols call for the rinsing of knives and glassware with such reagents as hexane before collecting tissues. Plastic containers are generally not useful for holding tissues to be analyzed for presence of hydrocarbons. Consult the toxicologist before starting a necropsy if toxins are suspected.

8. **Microbiology**: Have culturette tubes or sterile containers on hand to culture for bacteria or fungi. Consult the lab doing the culturing for materials and handling techniques. Use sterile technique when collecting samples. Glass slides should also be available for making impression smears of any lesions.

9. **Parasitology:** Containers of 95% alcohol, and glass slides and cover slips should be available for collecting parasites and examining mucosal swabs for parasite ova, respectively. Consult the lab doing identification: many will not accept parasites in formalin. Handle all parasites carefully taking care to not crush them as this may interfere with identification.

References/Resources

- Avian Necropsy Manual
  http://www.nwhc.usgs.gov/publications/necropsy_manuals/Avian_Necropsy_Manual-English.pdf
- Avian Necropsy Videos (chicken & duck)
  http://www.partnersah.vet.cornell.edu/Avian-Necropsy-Examination
- Wildlife Necropsy Videos:
  - PPE for Wildlife Disease Investigation
    https://cwhl.vet.cornell.edu/resource/ppe-wildlife-disease-investigation-and-response
  - A Guide to Avian Necropsy | LafeberVet
    https://lafeber.com/vet/a-guide-to-avian-necropsy/
  - Handling and Shipping Wildlife Specimens | Cornell Wildlife Health Lab
    https://cwhl.vet.cornell.edu/resource/handling-and-shipping-wildlife-specimens
  - Wildlife Health Program Webinar: Wildlife Forensics | Cornell Wildlife Health Lab
    https://cwhl.vet.cornell.edu/resource/wildlife-health-program-webinar-wildlife-forensics

Forensic References

- Huffman, J.E. & J.R. Wallace, eds. 2012. Wildlife Forensics: Methods and applications. Wiley-Blackwell: Hoboken, NJ
- Neme, L.A. 2009. *Animal Investigators: How the world's first wildlife forensics lab is solving crimes and saving endangered species*. Simon & Schuster: New York, NY
- Stroud, R.K. 1998. Wildlife Forensics and the Veterinary Practitioner. Seminars in Avian and Exotic Pet Medicine, 7: 182-192.
- Walker, D.N. & W.J. Adrian, eds. 2012. Wildlife Forensic Field Manual, 4th ed. Assn. Midwest Fish & Game Law Enforcement Officers, Colorado Parks & Wildlife: Denver, CO