Small Animal Necropsy Workshop

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With excerpts from the Wildlife Health Investigation Manual (Author: K Rose)
Available from:
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Post Mortem Examination

Post mortem examination (or necropsy) of animals can tell us a lot about their nutritional status, reproductive status and the diseases that they have contacted.

A necropsy is best conducted on an animal that has recently died. If you are unable to examine a freshly dead animal right away, keep it as cool as possible and away from predators and flies. Do no freeze the carcass if it can be avoided. Animals are fresh and suitable for necropsy when they are not bloated, discoloured, and their fur or feathers do not pull out readily.

**Equipment Needed**
- Gloves - vinyl, latex or clean dish-washing gloves
- Sunscreen
- Insect repellent
- Clean knife and steel to sharpen the knife
- Scalpel
- Clean forceps
- Sharps disposal container
- Sterile sample container (most often with a yellow top)
- 10% buffered formalin in leak proof plastic containers
- Leak-proof zip-lock bag
- Sample submission form
- Pencil
- GPS unit
- Camera
- Esky with ice or ice block for sample transport
- Hand washing soap, water, paper towel, OR alcohol based wipe

**Procedure**
- Confirm the species of animal. If you are uncertain, retain the carcass frozen after the necropsy and then consult a naturalist.
- Review the history of the animal, if any is known.
- Examine the outside of the body for any wounds or abnormalities.
- Examine the mouth, hooves, mammary glands, eyes, ears, anus for any blisters, ulcers, or other abnormalities.
- If asked to do so, complete any body measurement forms (morphometrics analysis).
• Open up the skin from the chin, down to the anus.

• From the centre incision, start to cut and pull the skin back around the body - towards the spine.

• When you get to the armpit, keep your knife under the shoulder blade to help pull back the front leg, in animals with a clavicle, cut through this bone.

• At the groin, cut through the hip joint to pull the leg back. Note the colour and consistency of the joint fluid.

STOP - assess the animal’s body condition: hydration (are the tissues moist or dry), muscle mass, and fat deposits

• Gently cut through the muscle of the abdomen following along the back edge of the ribs - DO NOT CUT THROUGH THE GUTS. The guts contain lots of germs, which will be released into the tissues if cut.

• Remove the abdominal muscles by cutting down the midline, following along the line of the ribs, and then along the spine. DO NOT BEGIN TO TOUCH THE TISSUES WITH YOUR HANDS AT THIS POINT.

• Cut through the diaphragm (You should watch closely to see the diaphragm collapse and you may hear the air rush in).

• Cut through the soft part of the ribs near the midline of the chest, near the sternum. Break or use bone cutters
to cut the ribs off, close to the spine to remove half of the ribcage. Smaller animals can be laid on their backs, cut the ribs off the entire rib cage by cutting all ribs as they approach the spine.

- Cut through the muscles along the inside margins of the bottom of the lower jaw bones to find the tongue. Pull the tongue out through the bottom of the jaw and continue to cut the tissues around the tongue, windpipe and throat to free them up but do not cut them off.

STOP - assess the animal's condition and look for anything unusual.
Initially, just have a good look over the organs (and try not to spread germs over them with your hands).
Wash your gloves to remove the hair and debris from the outside of the body before handling the internal organs.

Look for any lesions, which are changes in:
- Size, number
- Shape
- Colour
- Texture
- Lumps - firm lumps or lumps containing pus
- Soft spots
- Changes in smell
- Bleeding throughout many tissues
- Or the presence of parasites or foreign bodies in tissues

If you find any lesions:
- Get clean forceps and scalpel.
- Cut the lesion out of the organ, taking some of the surrounding tissue
- Cut the lesion inside the body or on a clean cutting board. Do not get dirt or hair on the instruments or tissues
- Cut the lesion in half.
- Put half of the lesion into the formalin container (alone or with other tissues). Make sure that this sample is small and not more than 1 cm x 1 cm x 3 cm
- Put the other half of the lesion into a sterile vial (by itself)
If the animal appeared sick, then collect:

<table>
<thead>
<tr>
<th></th>
<th>Formalin</th>
<th>Sterile Vial - to Freeze</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion</td>
<td>Half</td>
<td>Half</td>
</tr>
<tr>
<td>Gut content</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Liver</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Spleen</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Kidney</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Heart Blood</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Heart Muscle</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Tonsil or lymph node</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Now start to systematically examine and take samples from each organ. First look at the outside of the organ, and then feel the organ for any lumps, bumps or irregularities. Cut through the organ in several places to look for changes inside.

- Lungs - cut through the windpipe and look inside. Gently feel both lungs for any hard or soft lumps, then cut into each lung. There can often be bleeding in the lungs as a part of being shot or killed.
- Heart - collect heart blood if it is needed by the lab or if the animal appeared sick. Cut through the heart and wipe away extra blood with your knife to see the colour of the muscle.
- Liver
- Kidneys
- Spleen - found in white tissues beside the stomach
- Tonsils or lymph nodes (monotremes, birds and lower vertebrates don’t have these)
- Reproductive organs

Once you have looked at all of the solid organs, you might want to cut open the gut and look through. It is important to leave this part until the end to minimize the smell, reduce the number of flies, and prevent gut germs from getting on the other organs.

It is important to collect the stomach content if there is a mass mortality or if you suspect poisoning.
If the animal had behavioural abnormalities, remove the head and put it in a labelled bag to transport back to the lab. Remove the head by using a sharp knife to cut through the tissues between the base of the skull and the first neck bone. Find this spot by holding the nose and moving the head back and forth with one hand and using the other to feel along the back of the throat for the first feeling of movement. It is very difficult to cut between the first and second neck bones, so if you have problems, feel again for any movement at the back of the throat, moving closer to the nose.

DON'T FORGET TO:

• Label the vials with the date, animal’s identification number, species, and tissue collected
• Store the formalin fixed tissues out of the sun
• Store the tissues in the yellow-topped vials in an esky with ice and take them to the lab as soon as possible.
• Complete a necropsy worksheet
• If unable to reach a laboratory within 24 hours, ring them to ask how they would like the sample handled and stored.
## General Approach to Post Mortem Examinations

### Summary

- Thorough history
- External examination
- Central midline skin cut (chin to anus), reflect skin
  
  **STOP**

- Examine fat deposits, muscle mass, hydration
- Expose internal organs
  
  **STOP**

- Clean your hands and get clean instruments
  
  Look at all organs
  Collect clean samples for microbiology or freezing back

- Collect half (or 1 cm wedge) of any lesion into formalin and place the other half into a sterile vial to be frozen for later use.

- Examine each organ system thoroughly, collecting 1 cm wide wedge of each organ into formalin

- Complete a necropsy worksheet

- Properly label, store and ship samples
Sample Collection

Collection and proper handling of appropriate samples during and after a post mortem examination greatly increases the chances of finding the ultimate cause of death. Sample collection consists of four components: Preparation; Collection; Storage; Shipping.

**Preparation**
Before initiating the post mortem examination, have appropriate materials on hand. These include:

- Sterile collection instruments
- Plastic pots with formalin - the larger the better and ensure the mouth is as wide as the rest of the container
- Whirl-Pak bags or new Zip-Lock bags and sterile tubes
- Sterile swabs

Label collection containers:

- Species, Identification, Date, Tissue
**Collection**

**Tissues to be frozen (for Microbiology and Toxicology testing)**
- Collect liver, kidney, lung, spleen, lymph node, a portion of any lesion and stomach content
- Collect tissues for microbiology as soon as the tissue is revealed, to prevent contamination
- Use sterile collection instruments
- Place each tissue in a separate sterile container (Whirl-Pak bag)
- Collect as large a piece as possible (preferably 5x5x5cm)
- Keep on ice until samples can be frozen

Some tissues lend themselves better to swabbing
- Joints - open in a sterile manner
- Meninges
- Serosal surfaces

**Tissues to be fixed in formalin (for Histopathology)**
- Collect a complete set of tissues from every animal (see chart below)
- Tissue must be no greater than 1cm thick in at least one plane to ensure proper fixation
- Fix in 10% neutral buffered formalin
- Formalin to tissue ratio should be 10:1

<table>
<thead>
<tr>
<th>Brain</th>
<th>Stomach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye (inject with formalin)</td>
<td>Duodenum with pancreas attached</td>
</tr>
<tr>
<td>Tongue</td>
<td>Jejunum</td>
</tr>
<tr>
<td>Lung</td>
<td>Ileum with caecum</td>
</tr>
<tr>
<td>Heart</td>
<td>Colon</td>
</tr>
<tr>
<td>Liver</td>
<td>Lymph node</td>
</tr>
<tr>
<td>Spleen</td>
<td>Skeletal muscle</td>
</tr>
<tr>
<td>Kidney</td>
<td>Skin</td>
</tr>
<tr>
<td>Bladder</td>
<td>Other lesions / tissues of interest (Gonads)</td>
</tr>
</tbody>
</table>
Storage

- Microbiology samples to remain frozen
- Do not freeze if in transport media - submit to lab immediately
- Keep formalin fixed tissues out of direct sunlight
- Formalin fixed tissues can remain in formalin, but antigen availability decreases with longer fixation (important for testing like immunohistochemistry)

Shipping

- Ship microbiology samples on ice
- Prevent multiple freeze/thaw cycles
- Ship microbiology samples separate from formalin fixed samples
- To transport fixed tissues, allow to fix for 24-48 hours, then pour off formalin, wrap in formalin soaked paper towel and seal in a plastic bag
- Always ship with 3 layers of packaging and according to transport regulations
Post Mortem Examination Report Writing

Thorough and accurate descriptions of post mortem examination findings are very important to allow yourself or another investigator put the whole picture together once all testing is complete. Long periods of time may pass between the post mortem examination and finalisation of the case during which time details may become foggy in your memory. Post mortem examination reports consist of four components: Animal description; Lesion descriptions; Morphological diagnoses; Comments.

**Animal Description**

Physically describe the animal

- Species/breed (if unsure of species, freeze carcass following examination and consult an expert)
- Sex
- Age
- Weight
- Morphometrics (specific measurements requested for certain species, e.g. sea turtles)

Identify the animal

- Very important in zoological collections
- Examine for tattoos, bands/ear tags, microchips

Assess body condition and hydration

- Body condition based on fat stores and muscle mass
- Hydration based on stickiness of subcutaneous tissues (after peeling back the skin)

Assess preservation state of the carcass

- Are the eyes cloudy?
- Green discolouration on abdomen
- Grey putty appearance of liver
- Scavenging - there will be no associated haemorrhage with damage inflicted after death

**Lesion descriptions**

Draw a picture of the change with words focussing on the following (and remember, a picture is worth a thousand words, so take many, but also write the descriptions).

- Size (use measurements, do not compare to other objects, such as a “football”)
- Shape
- Colour
- Consistency: friable, soft, firm, hard (bone is hard)
- Distribution (see diagrams below)
Describe tissues changes only using simple terms

- No need to comment on normal, unless there is a specific question regarding that tissue
- No "kitchen pathology" (it's just distasteful!)

**Lesion descriptions:**

**Distribution**

- **DIFFUSE**
- **FOCAL**
- **LOCALLY EXTENSIVE**
- **MULTIFOCAL & COALESCING**

**Morphological diagnosis (for those with veterinary experience, can be omitted for others)**

The morphological diagnosis consists of the tissue involved and the pathologic process occurring. There should be a morphological diagnosis for every lesion described. The following are a few examples:

- Inflammation in the liver = Hepatitis
- Broken thigh bone = Femoral fracture
- Fluid on the lungs = Pulmonary oedema

The morphological diagnosis should then be fleshed out by adding at least 3 modifiers:

- Distribution: diffuse, multifocal, locally extensive, focal, miliary, cranioventral
- Duration: peracute, acute, subacute, chronic
- Severity: mild, moderate, severe

Therefore, the final product might look like:

- Hepatitis - multifocal, chronic and moderate
- Femoral fracture - focal, acute and severe
- Pulmonary oedema - diffuse, peracute and moderate
Comments

- This is your opportunity to put the story together and speculate on what you think is happening.
- Ask questions here, even if they will be left unanswered for the time being.
- Discuss the case in relation to other cases.
- Make recommendations regarding further treatment and possible management.
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