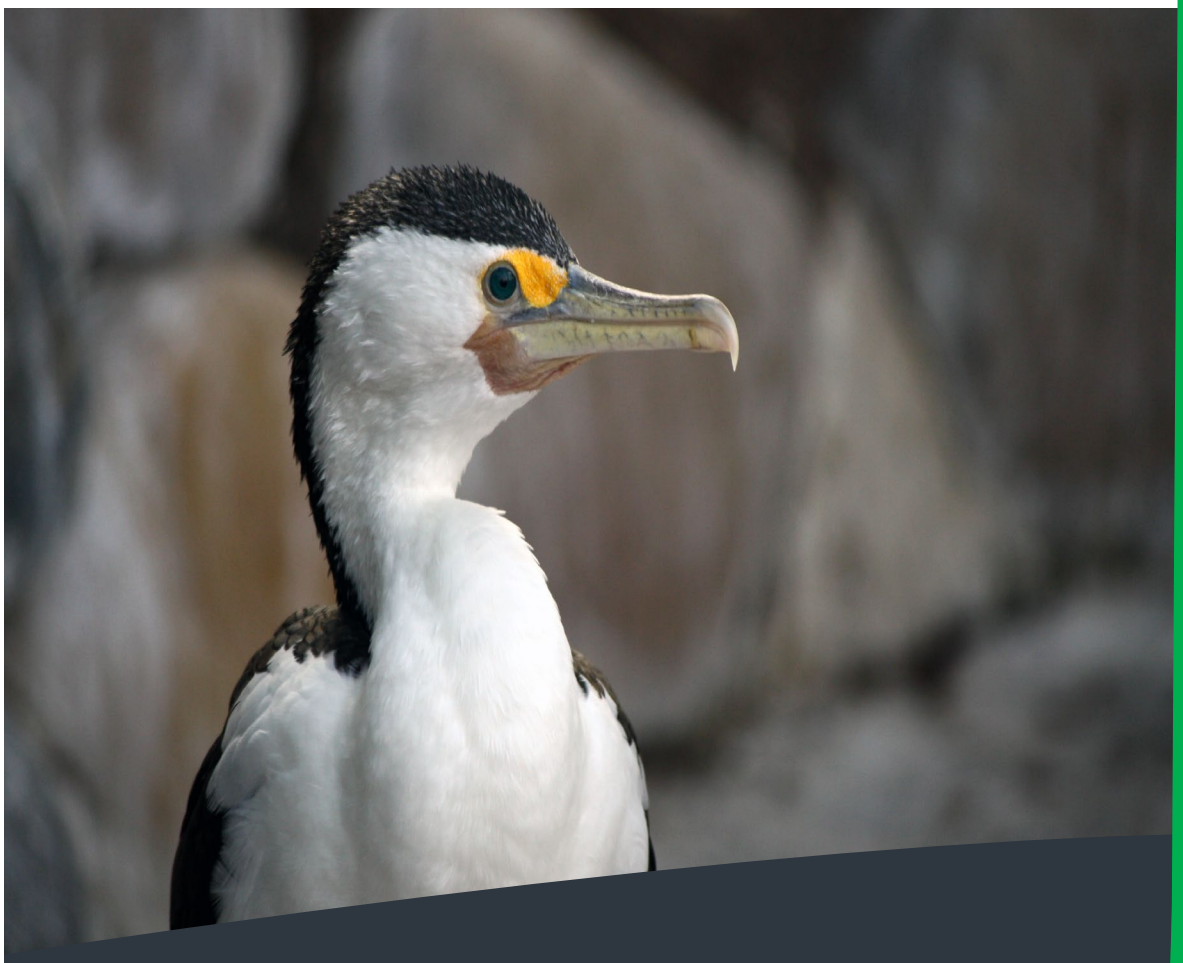
TARONGA
CONSERVATION SOCIETY AUSTRALIA

Australian Registry of Wildlife Health



Wildlife Health & Pathology Short Course 2019

Small Wild Animal Necropsy Workshop

Presented by:

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These notes draw from the content of the Wildlife Health Investigation Manual (Author: K Rose), available from:

The Australian Registry of Wildlife Health
Taronga Conservation Society Australia
PO Box 20
Mosman NSW 2088, AUSTRALIA
www.arwh.org

Your Health and Safety

This workshop will begin with a safety induction. Please remember that conducting wildlife necropsies is associated with risks, including, but not limited to injury from sharps, exposure to chemicals, and potential exposure to pathogenic organisms. When working with wildlife we must take responsibility for the safety of ourselves, our staff, students and volunteers. Consider starting and ending necropsies with a safety briefing if you are not alone.

Reasonable precautions to consider when planning to conduct a necropsy

Adequate hand washing facilities should be made available whenever there is direct contact between animals and humans. Hand washing is the single most effective tool in preventing the transmission of zoonotic agents and, thus, limiting the risk of infection.

Appropriate protective equipment to wear during a necropsy includes enclosed water-resistant shoes, a lab coat, latex or nitrile gloves, a waterproof apron, and safety goggles when sawing or using bone cutters. A P95/N2 respirator should be worn to necropsy bats, pinnipeds, primates and wildlife that had evidence of being thin or having respiratory or neurological disease.

The likelihood of injury from sharps can be reduced by ensuring that knives, scissors, and scalpels are maintained on a cutting board when not in use. All sharps should be returned to a specified cutting board and never left on the animal or on the ground. Scalpel blades should be removed with a specialised device. Knives and scalpels should be washed immediately after being removed from the cutting board and never left to wash or dry in a pile of instruments or at the bottom of a sink. Ensure that all assistants and observers stay out of the complete arc of your knife or scalpel blade. Never try to catch a falling knife or scalpel. Consider using cut resistant gloves. Knives should have a suitable guard or bolster to prevent your hand slipping down the handle to the blade. This is especially necessary for large animal examinations or for animals that have a large amount of fat or blubber which may make the handle slippery.

Human food should not be prepared, stored, or consumed near animal contact areas. Animal tissues should not be stored in containers, fridges or freezers that are used for human food. An old esky filled with ice can be used to chill animal remains prior to post mortem examination if a designated animal fridge is not available.

Immunocompromised persons, such as any individual with diabetes, chronic viral disease, chronic liver or kidney disease, persons taking immunomodulating drugs, splenectomised people, children, and the elderly should be discouraged from participating in a post-mortem examination.

Formalin and ethanol are common fixatives used in the collection of samples for further diagnostic testing. Both of these agents can be very caustic to exposed human tissues and ethanol can be flammable. Safety data sheets should be obtained whenever chemicals are ordered, and they should be accessible wherever the chemicals are used, stored, or transported. Spill kits should be available wherever chemicals are stored or used. Chemicals should be stored in bunded, purpose-built

cabinets.

A first aid kit and accident report form should be included in the equipment taken to any field site. Medical attention should be sought for any injuries sustained during the examination or illness that immediately follows the examination.

If a particularly dangerous zoonotic agent is considered as one of your differential diagnoses, consider referring the case to a specialist lab or conduct the necropsy within a biohazard safety cabinet. Limit the number of people exposed to the animal, necropsy and tissues emanating from the necropsy. Communicate your concerns with your state agriculture lab and anyone you send tissues.

Don't let this safety message deter you. Get in there. With each necropsy that you conduct you will become faster, more methodical, systematic, skilled and experienced. Post mortem examination is a wonderful tool to learn about natural history, anatomy, physiology and disease ecology in wildlife.

General Approach to a Post Mortem Examination

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. Guts last. ⇩
Complete a necropsy worksheet ⇩
Properly label, store and ship samples

Generic Small Wild Animal Post Mortem Examination Procedure

Post mortem examination (or necropsy) of animals can tell us a lot about their natural history including: nutritional status, diet, reproductive status, parasite burden, and exposure and impacts of potentially pathogenic toxins and pathogenic organisms.

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, nitrile, or clean dish-washing gloves
- Sunscreen
- Insect repellent
- Clean knife and steel to sharpen the knife
- Scalpel handle and blades
- Clean forceps
- Sharps disposal container
- Sterile sample container (most often with a yellow top)
- 10% neutral buffered formalin in leak proof plastic containers
- Leak-proof zip-lock bags
- Necropsy report and sample submission forms
- Pencil and clip board
- 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, take photos or retain the carcass frozen after the necropsy and then consult a naturalist.
- Review the history of the animal.
- 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.
- Complete body measurement forms (morphometrics analysis) that are relevant to the species.


- 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, tacky, or dry), muscle mass, and fat deposits.
- Change to clean instruments to avoid dragging fur, feathers or contaminants into the body cavities.


- Gently create a T-shaped incision through the abdominal muscles by cutting the tissue along the lower edge of the ribs, and then down the midline to the pubis. DO NOT CUT THROUGH THE

GUTS. The guts contain lots of germs, which will be released into the tissues if cut.

- Reflect or remove the abdominal muscles.
- Cut through the diaphragm. Watch closely to see the diaphragm collapse and listen to 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 the front half of the ribcage.
- 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

- Once again, 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 or instruments).
- Wash your gloves to remove hair, feathers or debris before handling the internal organs.
- Change to clean instruments to avoid contaminating 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
    - Or the presence of parasites or foreign bodies in tissues

- If you find any lesions:
    - Take photographs
    - Measure and take notes on the characteristics of the change
    - Get clean forceps and scalpel for each lesion
    - Cut the lesion out of the organ, taking some of the surrounding tissue
    - Cut the lesion inside the body or on a sterile Petri dish (clean cutting board). Avoid getting

dirt or hair on the instruments or tissues.

- Cut the lesion in half
- Put half of the lesion into the formalin container. Make sure that this sample is small and not more than 1 cm x 1 cm x 3 cm. Trim if necessary, but make sure that you have a representative section containing the margin of normal and abnormal tissue.
- Put the other half of the lesion into a sterile vial (by itself)
- Consider making an impression smear by pressing the cut section from the margin of normal and abnormal tissue against a glass microscope slide. Once dry, the smear can be stained with a Diff-Quik™ like stain solution and examined for cell type, cell morphology and presence of microbes. If you want to change to a gram or giemsa stain, simply place the slide back in the first Diff-Quik (methanol) to dissolve the stain.

- Proceed to observe the tissues and collect the sets of samples outlined below. Frozen tissues should be collected in a sterile manner so that microbes can be identified through culture or PCR.

	Formalin	**Sterile Vial (to freeze): Routine Necropsy**	**Sterile Vial (to freeze): Outbreak, Intoxication, Emergent Disease Syndrome**
Lesion	**Half**	**Half**	**Half**
Liver		**Yes**	**Yes** **Cryovial and large packet**
Kidney	**Yes**	**Yes**	**Yes** **Cryovial and large packet**
Brain	**Yes**	**Yes**	**Yes**
Gut content	**Samples of each level of the gut**	**Observe and describe**	**Yes**
Spleen	**Yes**		**Yes**
Heart Blood			**Yes** **Centrifuge and freeze packed cells and serum separately**
Heart Muscle	**Yes**		**Yes**

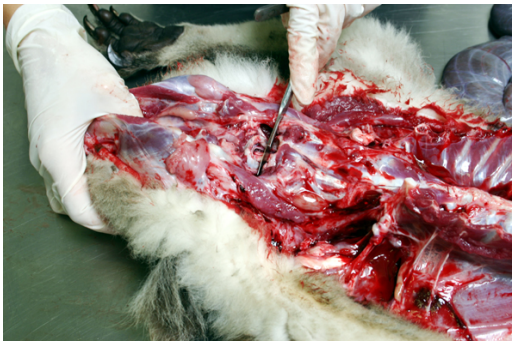
Tonsil or lymph node	**Yes**		**Yes**
Lungs, adrenal glands, thyroid glands, pancreas, gonads, mammary tissue, skeletal muscle, skin, peripheral nerve, spinal cord, bladder	**Yes**		

Once you have collected the tissues to freeze, start to systematically examine and take samples from each organ to be fixed in formalin. First look at the outside of the organ, and then gently 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 was not obtained prior to death, and is needed by the lab or if there may be value in conducting serological tests to see if the animal has been exposed to particular pathogenic organisms.
- Liver – examine each lobe of the liver for evidence of lesions including hard or soft lumps, or prominent or irregular colour change. Liver colour can change where it is in contact with the gall bladder. Then proceed to cut through the liver like a loaf of bread to look for further lesions and parasites within the large vessels.
- Kidneys - peel back the capsule (mammals)
- Spleen - found in white tissues beside the stomach
- Tonsils or lymph nodes (monotremes, birds and lower vertebrates don't have these)
- Reproductive organs, adrenal glands, thyroid glands, pancreas

Remove the head by using a sharp knife to cut through the tissues between the base of the skull and the first vertebral body. Find this spot by holding the nose and moving the head back and forth with one hand and using the other to feel the 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 skull.

Once the skull is removed the skin can be reflected and any fur or debris removed. Bone cutters or a saw angled along the occipital condyles or at a 45° angle to the spinal canal can be used to reflect the skull "cap". Get clean instruments to cut through the meninges and harvest a clear portion of brain into a sterile cryovial. Tip the skull upside down and starting at the foramen magnum gently cut away the cranial nerves along the roof and sides of the calvarium. Once you cut the olfactory nerves the brain should gently fall out for examination and fixation. Unless there are focal lesions, cut the brain in half along the long axis and fix an entire half. Use bone cutters or a saw to cut through the nasal cavity and sinuses. Collect segments of spinal cord, peripheral nerve and an eye to represent a greater proportion of the nervous system.

Once you have looked at all of the solid organs, cut open the gut and look at the mucosa and gut content. 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.

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.

Sample Collection

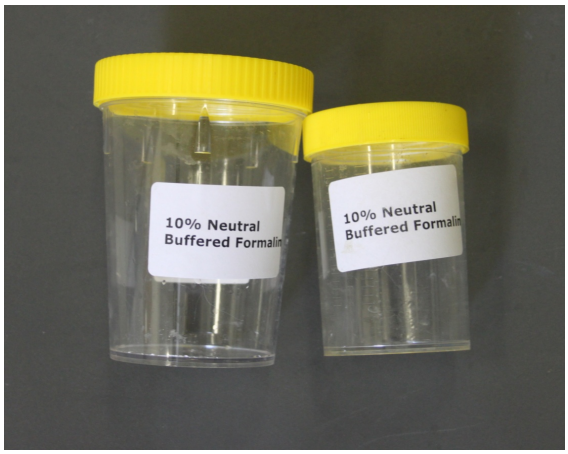
Collection and proper handling of appropriate samples during and after a post mortem examination greatly increases the chances of finding the ultimate cause of death. Sample collection consists of four components: Preparation; Collection; Storage; Shipping.

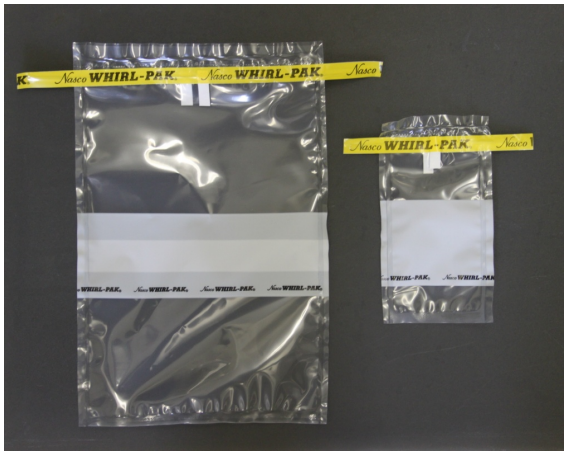
Preparation

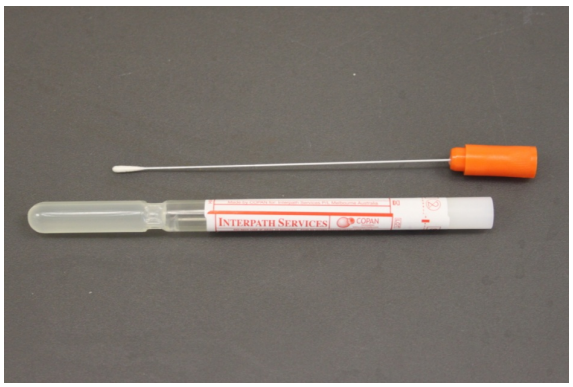
Chat with your diagnostic provider before collecting samples to ensure that you select an appropriate range of tissues, in the appropriate vials to meet your diagnostic goals.

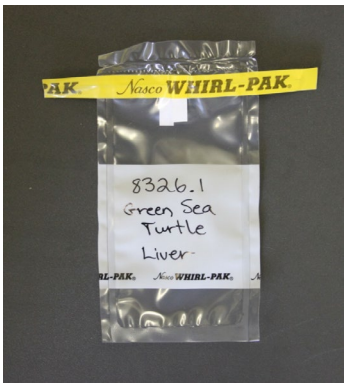
Before initiating the post mortem examination, have appropriate materials on hand. These include:

- 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 with Species, Identification, Date, and Tissue.

Collection

Tissues to be frozen for Microbiology and Toxicology testing

- Routinely collect and freeze 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 is a separate sterile container (cryovial, yellow top vial or 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 examined. Histopathology may not be conducted on each tissue in the first instance.
- Tissue must be no greater than 1cm thick in at least one plane to ensure proper fixation. Brain and lung are exceptions to this rule.
- Fix in 10% neutral buffered formalin.
- The volume of formalin in the vial should be at least 10 times that of the tissues to be fixed.
- Avoid narrow necked vials, as fixed specimens are inflexible and may be difficult to retrieve.

Photographs

- Specimen photography is an art and a science. Images are very helpful to communicate your findings with wildlife managers, diagnosticians, the public, and the scientific community.
- More information about specimen photography is available on the Registry website "A Good Picture is Worth a Thousand Words: A Practical Guide to Effective Gross Pathology Photography" prepared by Jane Hall.
- Important factors to keep in mind with specimen photography are:

- Background – black or dark grey preferably, non-glare, clean (no blood pools extraneous tissues, bloody gloves or instruments).
- Focus – take several photos, good focus is more important than getting close.
- Orientation – keep your camera perpendicular to the field and keep the field flat.
- Lighting – even across the field, glare reduced, multiple sources of light if possible to reduce shadow and glare.
- A ruler/scale with case number included on the lower right-hand margin (so that it can be cropped if necessary for publication).

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 and do not freeze
- Formalin fixed tissues can remain in formalin, but antigen availability decreases with longer fixation (important for testing like immunohistochemistry)
- Keep any blood films or impression smears away from formalin fixed samples

Shipping

- Ship microbiology samples on ice at the beginning of the week when possible
- 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. Ship in a box with rigid sides to prevent them being squashed, or containers being broken during transport.
- Always ship with 3 layers of packaging and according to transport regulations
- Keep a portion of the frozen samples in case the package gets lost

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.

Try to describe your findings sufficient for another person to visualise the remains and form their own interpretation of events preceding the animal's death. Be sure to include body condition, the volume and nature of ingesta, and reproductive status in your descriptions.

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
- Morphometric data (specific measurements requested for certain species, e.g. sea turtles)

Animal identifiers

- Very important to check and document in zoological collections and trapped wildlife
- 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)

Classify and describe the state of preservation of the carcass

Class 1 - Live animal. Optimum specimen for examination and testing. The animal's behaviour can be monitored, blood can be collected and then the animal can be euthanased for post-mortem examination.

Class 2 - Fresh = Recently dead. Good specimen for examination and testing.

Class 3 - Mild to moderate decomposition, but organs still intact and recognisable. There may be mild bloating of the carcass as the intestines fill with gas. The tissues of the mouth and eyes may be dry and wrinkled, but are not green or purple. The fur or feathers do not pull out easily. Examination of these animals can provide useful information, but the tissues are less suitable for additional testing than those in class 2.

Class 4 - Advanced decomposition with organs no longer intact. The carcass is usually bloated and foul smelling. The oral tissues are often green or purple. The fur or feathers are easily removed with gentle traction. The internal organs are soft or liquefied and the intestinal tract is distended with gas. A gross post-mortem examination may provide some useful information, but tissues are generally unsuitable for further diagnostic testing other than heavy metal analysis.

Class 5 - Mummified or skeletal remains. Only gross, radiographic and genetic examinations are possible.

Lesion descriptions

Photograph or draw a picture of unusual or complicated changes, but use those to help illustrate rather than replace your description.

- 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

- There is no need to comment on normal findings, unless there is a specific question regarding that tissue
- Please don't compare tissues with common foods

Morphological diagnosis

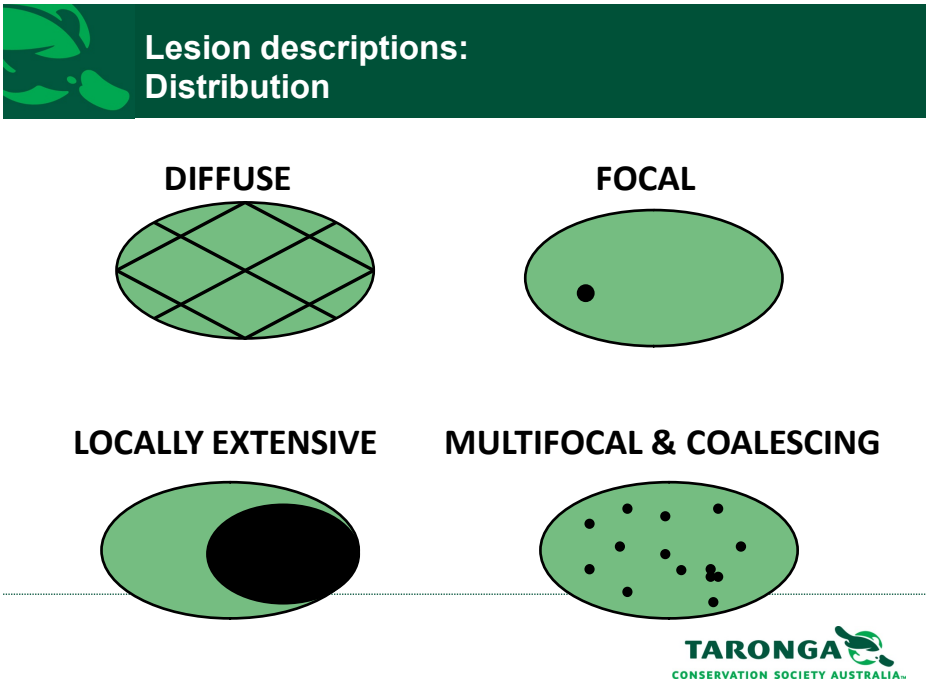
For those with veterinary experience, can be omitted for others.

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

Report Comments

This is your opportunity to put the story together and speculate on what you think is happening. Feel free to pose questions, even if they will be left unanswered for the time being. Try to discuss your findings in light of the interest of the submitter and other readers. Make recommendations regarding further treatment and possible management.

TARONGA CONSERVATION SOCIETY AUSTRALIA

Australian Registry of Wildlife Health

OFFICE USE ONLY

Case Number:

Post Mortem Examination Form

Submitter Information

Organisation - _____

Contact name - _____

Address - _____

_____

Phone: Work _____ Home _____

Mobile _____ Fax _____

Email _____

Date Submitted: _____

Your Ref No.: _____ Lab Ref No:_____

Animal Information

Species (common or taxonomic name):

_____

Animal ID and type:_____

ARKS/Rehab No: _____

Animals given name: _____

Sex: _____

Age: Adult/Juvenile/Pouched Young/Nestling/Foetus

Approximate age: ____ Yrs / Months / Days (circle)

Weight: _____ g / kg

Where animal was held:

Zoo/Private/Wild/Rehab/Quarantine/Feral (circle)

Location name (e.g. town/locality):

_____

If zoo, enclosure ID/description: _____

Epidemiology

Number of Animals Dead: _____

Number of others affected/sick: _____

Date Animal Died: _____

Death circumstances: euthanasia / found dead / treated & died / live - biopsy / died in transit

State of specimen: fresh/mild decomposition/ moderate decomposition/advanced decomposition

Samples submitted: carcase/blood/tissue/serum/egg/foetus

Case History, e.g. where animal was located, state of animal when found, previous medical records or history of animal etc.

_____

_____

_____

_____

_____

Necropsy Date: _____ Prosector: _____

Gross Post Mortem

External Findings: _____

_____

_____

_____

Hydration: _____

Fat Deposits: _____

Muscle Mass: _____

Stomach Content: _____

Internal Findings: _____

_____

_____

_____

_____

Cytology /Microbiology Findings: _____

_____

_____

_____

Diagnoses:

_____

_____

_____

Comments: _____

_____

_____

Materials Saved	
Researchers	Radiographs
Paraffin Blocks	Serum
Microscope Slides	Photographs - gross
	Photographs - microscopic

NOTES: