COLLECTION OF NECROPSY SPECIMENS FOR LABORATORY INVESTIGATION

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Necropsy and follow-up laboratory investigation are essential procedures for accurate disease diagnosis in all animal species. The procedures are particularly important where there are numbers of animals at risk or where disease prevalence is poorly documented in the species or animal group being investigated. Prior to undertaking necropsies one should be aware of the risk of zoonoses to the operator. Of particular concern is psittacosis (Chlamydophila psittaci), found widely in psittacine birds and occasionally in other bird species. Within the laboratory, necropsies of psittacine birds are routinely conducted in a biohazard cabinet to avoid the risk of being infected by inhalation. Necropsy of flying foxes is also of concern due to the prevalence of Lyssavirus in these animals. Most laboratories require veterinary pathologists to be vaccinated for rabies if flying foxes are to be necropsied. The risk of avian influenza occurring in Australia is generally considered to be low but this may be another consideration, particularly when examining shore birds. A range of other potentially zoonotic infections occur in Australian wildlife so in many instances it would be wise for non-veterinarians to forward the animal to a diagnostic veterinary laboratory via your local veterinary clinic or wildlife authority.

Forwarding animals for necropsy

Animals should be refrigerated as soon as possible after death and forwarded to the laboratory on ice within a cooler. Bodies usually remain well-preserved for up to 3 days under normal refrigeration (4°C), but there may be some degradation of sensitive tissues such as intestine within hours of death. Freezing should be avoided since it damages tissue structure used for histological examination and may affect the viability of some micro-organisms.

Post mortem technique

In situations where an animal cannot be forwarded to the laboratory within a reasonable time of death the following techniques should be helpful in obtaining a diagnosis.

a) Equipment

The equipment and expendable requirements for a well-conducted necropsy are as follows: Protective rubber gloves, a narrow-blade sharp knife or scalpel, sharp scissors with pointed tips, forceps, bone cutters (foot-trimming shears, garden shears or branch loppers are useful), running water or a bucket of water, a portable gas jet (for sterilisation of instrument tips), sterile plastic 70ml ('yellow top') specimen containers, a container of 10% buffered formalin (allow at least a 5:1, preferably 10:1 formalin:tissue ratio). A coarse hacksaw may be required for removal of the brain from medium sized to large animals. Swabs with transport media can be useful for culture of difficult to sample sites but in most instances a solid tissue sample or fluid sample is preferable to a swab. Sterile syringes and 19G needles or vacutainers may be useful for sampling body fluids. A surgical mask and goggles may be useful in reducing exposure to potential zoonotic agents (e.g. if psittacines are autopsied). A bucket of warm soapy water is helpful for wetting bird plumage prior to necropsy. It is valuable to have someone take notes during the necropsy.

b) Partial necropsy

A full or partial necropsy may be performed depending on the size of the animal. Since gross and histological examinations by a pathologist are usually the most important procedures for diagnosis, small animals should be partially necropsied and entirely fixed in formalin for further laboratory investigation. Trim to reduce size by removal of tail feathers and possibly limbs, then open the abdomen, thorax and cranium to allow fixation. It is useful to collect fresh samples of some tissues – typically liver (sample first), any visibly abnormal organs and intestine (sample last) - prior to immersion of the body in formalin. Be sure to heat sterilise the tips of sampling instruments using the gas jet before taking each fresh specimen into separate containers. For best fixation of the viscera in formalin it is useful to exteriorise these from the abdomen and thorax by severing attachments to the underside of the spine. The digestive tract may also be partially opened to allow formalin penetration by using sharp tipped scissors to snip into the

stomach/gizzard and slitting along a 1-2cm section of intestine in at least two places. The brain should be exposed to formalin by snipping off a dome of cranial bone using a small pair of sharp scissors. If formalin volume is insufficient to fix the entire animal consider formalin fixation of the entire abdominal and thoracic viscera (including oesophagus and trachea to the level of the larynx) and the head/neck. Note that in birds and reptiles the kidneys are closely attached to the underside of the spine and these will need to be removed separately for fixation.

c) Full necropsy

For a full necropsy, initially perform a thorough external examination of the body. Note any external parasites and consider collection of these for identification. Note any wounds, swellings, ulcers or other abnormalities and consider sampling into formalin. Note that tissues sampled into formalin should be of limited size, generally specimens of 2-4cm in maximum dimension and 10mm maximum thickness for medium to large animals and smaller specimens (5-15mm maximum dimension) for birds and small mammals. Orifices should be examined for any abnormalities; any discharges should be noted. Mucous membranes should be examined for colour (pallor, jaundice, cyanosis) and the presence of other abnormalities such as haemorrhages or erosions.

The body is then prepared for internal examination. With birds and other small animals it is useful to wet the feathers/fur by partially immersing in warm soapy water then rinsing with plain water and partially drying with paper towel. This reduces contamination of internal organs and gloves with feathers or fur. A ventral midline incision is made through the skin from the upper neck to the posterior abdomen and then dissected back to expose the underlying muscles. Also remove skin from the inner thighs and sample muscle into formalin. The nutritional condition of the animal should be noted – muscle atrophy, presence of subcutaneous fat (note that wildlife is usually very lean) and bone strength/rigidity. Further observations on nutritional condition can be noted when the abdomen and thorax are later opened. The mouth should be examined. For small animals the angle of one side of the jaw/beak should be cut with scissors to expose the pharynx and larynx. The cut through the angle of the mouth can then be continued from the pharynx down the oesophagus. In seed eating birds the crop (an outpouching of the oesophagus) will be encountered in the mid neck region. The contents of the crop should be noted and removed (or sampled if abnormal). The pharynx, the larynx and the crop are important sites for infectious diseases in birds. Any thickening, plaque formation or other abnormalities in these areas should be noted and the tissues sampled into formalin.

Next the instruments should be washed and heat sterilised before opening the abdomen and thorax. The abdomen is opened with a scalpel or sharp knife by incising the abdominal muscles in an arc following the posterior edge of the ribcage while grasping and lifting the abdominal muscles with forceps as the incisions are continued down the lateral edges of the abdomen towards the pelvis. The liver is often a useful organ to immediately aseptically sample into a sterile for possible microbiology or toxicology. Spleen, kidney and pancreas may be useful for viral testing but this is rarely required. If other organs or membranes appear abnormal consider sampling these with sterile instruments before opening the digestive tract. Also before opening the digestive tract the thorax should be examined.

To examine the thorax use scissors, shears or loppers (depending on the animal size) starting under the abdominal edge of the rib cage and continuing forward on each side. The lungs are normally bright pink and the heart normally has only a trace of fluid in its enclosing sac. Specimens of lung and heart are normally routinely sampled into formalin. For small animals fix the entire heart in formalin; for larger animals open the ventricles and examine the heart muscle and valves.

Returning to the abdomen, the entire viscera may be removed (small to medium animals) or examined in situ (medium to large animals). Sample liver, spleen, kidney, adrenal and gonads into formalin then open the digestive tract. Note the contents of the stomach/gizzard/proventriculus and consider sampling these (unpreserved, frozen) if poisoning or botulism is suspected. Add tissue samples to formalin. Note the contents of the intestine and formalin-fix a sample of the upper intestine with attached pancreas. Fresh intestine may be useful for

bacteriology. Lower small intestine, the caecum (if present) and the large intestine should be routinely sampled into formalin. The brain should then be removed or the head fixed in formalin with the brain exposed by removal or the cranium. The cranium can be removed in birds and small animals using sharp scissors or pointed shears. In larger animals a hack saw will be required, either halving the brain with a longitudinal vertical cut through the skull or by removing the cranium with a transverse cut behind the eyes and an angled longitudinal cut on both sides.

Specimen storage and transport

Non-preserved specimens should be refrigerated and should preferably reach the laboratory within 24 hours but will generally remain useful for up to 72-96 hours. Formalin-fixed specimens can be held at room temperature and are useful for an indefinite period after sampling. A detailed history and necropsy notes should be included with specimens sent to the laboratory.