Hygiene Protocols for the Prevention and Control of Diseases (Particularly Beak and Feather Disease) in Australian Birds

Full Necropsy Protocol

Australian Government

Department of the Environment and Heritage

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Disclaimer

Note

This document describes a Full Necropsy Protocol. It has been developed with the involvement and cooperation of a broad range of stakeholders, but the making of this document does not necessarily indicate the commitment of individual stakeholders to undertaking any specific actions. The attainment of objectives and the provision of funds may be subject to budgetary and other constraints affecting the parties involved. Proposed actions may be subject to modification over the life of the document due to changes in knowledge.

Full Necropsy Protocol (Captive Bird)

Introduction

It is important that personnel who may be performing necropsies undergo training in the technique, including specimen collection and how to submit them. This is especially important now that avian influenza has become a significant threat.

While conducting your necropsy, you will look for changes in size, colour, shape and texture of organs and the presence of foreign bodies.

Occasionally, carcases may need to be frozen. In these circumstances, and after recording the findings of a full inspection of the skin and plumage, wet the plumage with a 1% detergent solution, placed in two sealable plastic bags, and store at -20°C or less. The detergent allows the liquid to penetrate the feathers, preventing them acting as insulation and so delaying the cooling of the internal parts of the carcase. Identify each frozen carcase with a number, sex, whether adult, sub-adult, young or neonate, the dates of collection and of freezing, the findings from inspection of the skin and plumage, and any signs were observed prior to death (if the bird was not found dead).

A necropsy needs to be undertaken:

- to determine why an individual bird has died
- to collect specimens from a bird or birds when it is known what caused the death(s)
- to investigate multiple mortalities in one species in an area
- to investigate multiple mortalities of more than one species in an area

A necropsy may be undertaken with assistance. If assistance is not available, then the person conducting the necropsy should have a hands-free voice recorder to record all observations. Begin recording just prior to donning disposable gloves, and place it in a secure pocket. Ensure that the microphone does not interfere with your mask. Never touch the recorder while you are wearing gloves. Alternatively, you may rely on your memory and fill in a checklist at the end of the necropsy.

If images need to be taken, and assistance is not available, then disposable gloves are to be removed and the image taken. Under no circumstances is the camera to be handled with disposable gloves.

Human Health

Zoonoses

Some avian diseases of Australian native psittacine birds are transmissible to humans (eg, *Chlamydophila psittaci*, Salmonella spp, rarely influenza type A, certain subtypes). Take care when doing a necropsy on a native bird:

Safety

Where will the necropsy occur? Safety precautions are more easily implemented in a designated necropsy room, then in the field. If in the field, ensure that you can adopt a comfortable standing position and have a rigid surface on which to place the bird.

Do not contaminate the environment with infectious material, or contaminated equipment.

Formalin

- Formaldehyde is used to preserve tissues for histopathology and is a potential carcinogen that may cause illness in later life from excessive uncontrolled exposure
- Avoid unnecessary exposure to formalin. Good ventilation is vital. Avoid inhaling the vapour.
- Be aware of the carcinogenicity of this chemical, and to exercise care when placing samples in it.
- Never pour formaldehyde down a sink. Take precautions to prevent contaminating the environment with formaldehyde.
- Return unused formaldehyde to a central location for disposal.

History

Record

- The location
- Species involved:
  - adult, sub-adult, juvenile, neonate, egg
  - male, female, sex undetermined
- Number of animals involved
- Clinical signs.
- Take images, preferably with a digital camera, being very careful not to contaminate the camera.

Euthanasia

The intravenous administration of sodium pentobarbitone (100 mg/kg) is preferred. Dilute 1:1 for small birds and 1:3 for very small birds to avoid artefactual changes in the vessels and heart due to pentobarbitone toxicity.

Cervical dislocation is an acceptable method for birds weighing less than 1 kg. The operator must be able to perform this procedure quickly and effectively, so that consciousness in the bird is immediately lost.

Note

Birds lack a diaphragm and the cavity housing the viscera is referred to as the coelom. However, the terms “thorax” and “abdomen” will be used in this document to refer to the areas that are separated by the diaphragm in mammals. In some cases the term thoracoabdomen may be used, meaning the coelom, or both the thorax and abdomen.

Procedure

If you are unsure of what has killed the bird, or if it has been presented as "sick", then it is important that a complete range of tissue and blood samples be obtained from the carcase. If only a selection of samples is obtained (eg, because a particular disease is suspected), then other diseases (which require other samples to exclude their presence) cannot be excluded. If you know the bird was sick, or died from a particular disease, then you may take appropriate samples for that disease. However, always be guided by the laboratory to which you will be submitting samples.

The necropsy must be performed as soon as possible after death to avoid decomposition of internal organs. Always indicate if samples are sterile or non-sterile

External examination

After donning protective clothing (see Equipment Lists) note the species, sex and weigh the bird
Note leg band numbers, if present, or other identification

If you need to take morphometric measurements, ensure that the measuring equipment is metallic or plastic, so that it can be disinfected after the necropsy.

- A bird in good body condition has rounded, firm pectoral muscles and minimal subcutaneous fat.
- A prominent keel and wasted pectorals indicates weight loss.
- Fat birds often have large fat pads over the pectorals and protruding abdomens
- Palpate the legs and wings, feeling for fractures, dislocations, lumps or deformities.
- Palpate the joints for swelling
- Examine the skin, plumage, beak and nails
- In psittacine birds, look for presence or absence of powder down.
- Feathers should sit tightly, be clean, and not damaged or misshapen
- Is the bird moulting?
- Missing or mis-shapen feathers may indicate PBFD, APV infection or feather picking
- Signs of trauma
- Examine body orifices - eyes, nares, oral cavity, ear, vent for discharges, foreign bodies.
- Look for external parasites
- Examine the uropygeal gland (if present in the species)
- Examine the beak and nails for deformities, fractures or delaminations
- Are the nails normal, overgrown or distorted?

Wet the bird's feathers with a warm 1% detergent solution. This allows the water to penetrate the feathers and prevent powder down/dander floating and being inhaled or contaminating the viscera. Ensure that the head does not get wet (may compromise microbiology of eyes, ears, nares and mouth due to contamination). Alternatively, collect smears and microbiology samples from ears, eyes, nares, mouth and cloaca before wetting the carcase. If a bird cannot be necropsied immediately, soak it in 1% detergent solution, ensuring that any trapped air is squeezed from the plumage, wrap the bird in a sealed plastic bag, chill it in the freezer for 5 minutes and then place it in a refrigerator at 4-6°C until a necropsy can be performed.

Remove the feathers on the ventral surface. Waterfowl are very heavily feathered and so are difficult to pluck. You may need to soak the bird for a few minutes to allow the feathers to be parted without adhering to your gloves..

Head and Neck

- Place the bird on its back with its feet away from you.
- Examine the head and neck, looking for abnormalities in the eyes, ears, beak and oral cavity. Apparent haemorrhages in the skull can be a post mortem artefact.
- Examine the external auditory meatuses (ear openings and canal).
- Turn the head to the left of the bird, presenting the right side of the head and neck. Cut through the right lateral commissure of the mouth, continue the subcutaneous incision down to the thoracic inlet and over the sternum to the cloaca. Reflect the skin, exposing the oesophagus, crop, pectoral muscles and abdominal wall. Observe the thymic lobes which lie on each side of the neck, along each jugular vein. These may or may not involute as the bird ages. In the domestic fowl, thymic lobes are found at two and more years of age.
- Note that pigeons have a vascular plexus in the neck and that this can be mistaken for haemorrhage, if accidentally incised.
- Examine the oral cavity, tongue and choanal slit. Samples may be collected from the conjunctiva and choanal slit at this time.
- Make a longitudinal incision in the larynx and trachea. You may need to hold the tongue with forceps to start your incision. Describe any lesions.
- The tracheas of some birds (some waterfowl) are arranged in loops. This is normal. For example, in the magpie goose, the trachea travels subcutaneously down to the cloacal area, then returns to the thoracic inlet, crosses to the other side, goes back to the cloaca, then returns to the thoracic inlet and then branches into bronchi and enters the lungs.
- The syrinx of male ducks has an expansion on the left hand side called the tympanic bulla. The shellduck has one on both sides of the syrinx, the right being slightly larger. The whistling duck (*Dendrocygna* spp) has symmetrical bullae. These are normal.
- Make a longitudinal incision through the oesophagus and crop and examine any content, as well as the surfaces.
- Remove the upper beak with a transverse cut near the eyes (bone shears may be needed for this, depending on the size of the bird). Examine the nasal cavity and opening to the infraorbital sinus (under the eyes) for abnormalities. If pathology of the sinuses is seen, obtain a swab from inside the sinus.
- Examine the eyes. Swellings beneath the eyes (infraorbital sinus), indicate vitamin A deficiency.

- The eyes and brain will be looked at later.

Body

- Turn the bird around with its feet towards you
- Disarticulate the hips (coxofemoral joints) by forcing the knees outward. This helps to stabilise the carcase.
- If the bird is very small, the wings and legs may be pinned to a dissecting board to keep the carcase steady.
- The pectoral muscles should be turgid and brownish-red. Examine for decreased muscle mass or bruising. Look for pallor or streaking, which may be an indicate exertional myopathy or selenium/vitamin E deficiency. Whitish stripes in the musculature may indicate sarcosporidiosis. Serially incise the pectoral muscles, looking for lesions.
- If you are not performing the necropsy in a biohazard cabinet, cover the carcase with plastic or glad-wrap, place your hands underneath and incise the abdominal muscle from just in front of the cloaca to the sternal edge (this is to avoid inhaling the plume of aerosols that sometimes occurs when you open the abdomen). Ensure that you have penetrated the abdominal airsac before removing the covering.
- Remove the covering and extend your incision along the last ribs on each side up to the spine. Reflect the abdominal muscle backward to expose the abdomen.
- In most cases, the ribs may be cut along the line where they meet the spine. At this point, the ribs

are cartilage and are easily cut. Cut from the abdomen to the thoracic inlet, and remove the sternal plate, taking care not to tear the pericardial sac. If you wish to cut the ribs from the spine, you will need bone shears.

- The pericardium should be transparent. There should be no fluid within the pericardial sac. Examine the heart for discolouration, paleness, pale area, or "spangles"(visceral gout). Remove the heart and open it to examine the valves for lesions (like mammals, the avian heart has four chambers). The right atrioventricular valve is muscular, not membranous. The heart may be fixed without cutting in small birds.
- There should be fat around the top of the heart. If this fat is jelly-like, it indicates the bird may be mobilising its fat stores because it is not eating
- Locate the thyroid glands located at the thoracic inlet lateral and slightly behind the syrinx and adjacent to the carotid arteries. The right thyroid contacts the oesophagus. Examine for enlargement and locate the parathyroid glands, lying at the caudal pole of each thyroid gland.
- Proceed down to the sides of the liver.
- Examine the caudal thoracic airsacs, located behind each lung. A normal airsac should be clear, like glad-wrap in most species (it is often at least partially opaque in ratite birds and penguins) . If you can imagine you can read a page of print through the airsac, then it is normal. If abnormal, obtain a swab and sample. Place the airsac sample in a container immediately, since it may be difficult to find if left on the bench surface.
- There is often a large amount of fat around the cloaca, especially in waterfowl.
- The thoracabdominal cavity should not contain any fluid. The presence of fluid or fibrin should be noted and a swab taken.
- Examine the liver for changes in size, colour, white or yellow spots, abscesses, tumours. The liver should be a mahogany brown. The shape and size varies between species - the lobes appear approximately the same size in raptorial birds and domestic fowl. The right lobe is larger in psittacine birds. In birds that eat fish the right lobe is larger and may extend to the cloaca. The gall bladder should be examined (it is absent in some species). Many mistake the liver for the lungs.
- A swollen yellow fragile liver is normal in neonates - the liver is infiltrated by fat absorbed from the yolk sac. The liver attains a normal colour during the second week of life, although this depends on the size and species of bird. A fatty liver is also normal in a laying bird, and the liver returns to a normal colour 7-14 days after laying the last egg.
- In young birds (usually less than 5-7 days old), the yolk sac will be seen when the abdominal cavity is opened. This is attached to the umbilicus at one end, and the mid-point of the small intestine at the other (Meckel's diverticulum). It is enclosed by the abdominal wall just prior to hatching and is a store of food for the neonate during the first few days of life.
- Observe the abdominal viscera before you disturb it. There will be minimal fluid in a healthy bird. Excessive amounts of fat are abnormal, except in an indeterminate laying bird. Note any excessive exudate or fibrin. Yolk maybe present if a follicle and yolk has not been engulfed by the infundibulum and has entered the abdominal cavity. (Determinate layers are birds which lay a specific number of eggs per nesting. Indeterminate layers are birds which will lay extra eggs if some are removed from the nest during incubation. A domestic fowl is an indeterminate layer).
- Grasp the proventriculus and rotate it to your left side (i.e., the bird's right side). This exposes the spleen, caudal to the proventriculus. The spleen is oval in psittacine and galliform birds, comma-shaped in passerine birds, and sausage-shaped in columbiform birds. Note any abnormality.
- Remove the liver, proventriculus, ventriculus (gizzard), small intestines, large intestine, caeca (if present), and bend the viscera caudally, leaving the cloaca attached to the body. The cloacal bursa (in a young bird) lies dorsal on the cloaca..
- The pancreas can be seen as pinkish organ cradled within the loop of duodenum the first part of the small intestine.
- A green discoloration of the liver near the gall bladder is a normal finding.

- The adrenal glands are located on both sides of the abdominal aorta close to the medial border of the cranial pole of each kidney. They may be covered by the testicles in the male, while the left one is covered by the ovary in the female.
- Male birds have two testes, located just beneath the vertebral column near the cranial pole of each kidney. The testes vary considerably in size from small during the moult to large at the height of the reproductive cycle. They also vary in colour between species, from pearly white to dark green or black.
- In the female, the ovary lies against the cranial pole of the left kidney. Quiescent ovaries are finely granular, while during egg production the ovary is very large, covering the cranial and middle lobes of the left kidney and containing many developing and mature follicles.
- The paired kidneys lie in the synsacral fossa on each side of the vertebral column. Each kidney is elongated and consists of three successive lobes joined together by bridges of kidney tissue. The colour of the kidney varies from pink to brownish red, depending on the blood supply. In very small birds it may be impossible to remove the kidneys without fragmenting them, so it may be necessary to cut them out attached to the spine.
- The lungs may now be seen, two bright pink and small (compared with mammalian lungs) organs located under the vertebral column and extending from the first to the last rib. These may be gently teased from the rib cage. The lungs should be removed because many lesions occur on the hidden dorsal and lateral surfaces.
- Look for enlargement of the sciatic nerves located on the interior upper thigh and near the medial side of the femur
- Incise the hip, knee and hock joints, looking for abnormal exudate - the joints should be smooth with only a small amount of clear fluid. Incise the tibia and tibiotarsus looking at the bone marrow.
- The cloacal bursa (bursa of Fabricius) lies on the dorsal aspect of the cloaca. This organ disappears when the bird reaches sexual maturity.
- Now look at the gastrointestinal tract, starting with the proventriculus. Incise it lengthwise. The normal surface should have pore-like nodular thickening - these are the openings of the submucosal glands. Collect a smear for Avian Gastric Yeast.
- Incise the gizzard, intestines and caeca (if present). The gizzard of seed-eating and omnivorous birds is thicker than that of carnivorous and piscivorous birds. The latter is thin and blends with the wall of the proventriculus. Examine the gizzard contents for foreign bodies or heavy metal fragments. Note the appearance of the inside walls (mucosa) and the presence of parasites, or thickening of the surface. Tease the gizzard lining from the underlying muscle - parasites commonly occur there. The caeca may contain blood, pus or necrotic material. Smears should be made of the small and large intestine and caeca (if present) and examined for parasites and coccidia. Deep scrapings should be made to look for *Capillaria* spp.
- Return to the head. Secure the head and peel the skin of the scalp forward to expose the skull. Small areas of bluish blood are sometimes seen here, and usually do not indicate trauma. The brain may be removed by gently incising the bone at the front, and tipping the head upside-down so that the brain drops away as its attachments to the cranial nerves are cut. This is more difficult in psittacine birds, because of the strong bone plate that covers their optic lobes.
- Remove and inspect both eyes. The eyes are much larger than they appear, and must be removed by dissection of the soft tissues and section of the lateral wall of the orbit. The optic nerve is cut and the eye removed.
- The brain is more easily removed in small species. If a swab is required, the sterile swab may be inserted through the foramen magnum. The skin over the dorsum of the head is removed, exposing the bone beneath. The cranial bone is very thin and can be incised to expose the brain below. Inspect it for haemorrhages or congestion. For smaller birds, hold the skull upside-down and allow the brain to exit the cranium without forcing, while severing the cranial nerve attachments. The skull of small birds may be placed in fixative and the bone decalcified later. Alternatively, the head and brain may be placed in fixative after the calvarium (bony covering

on the top of the brain) is removed. The head may also be transected longitudinally and both halves placed in fixative. Large brains should be partly sliced to enable the fixative to penetrate more easily, and the fixative should be renewed every day for 2-3 days. The spinal cord may be removed by blunt dissection and placed on a piece of card for fixation.

- Dispose of the carcase and disposable gear properly and disinfect all surfaces and instruments.
- Record all necropsy findings thoroughly.

Further Reading:

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