

Diagnostic Value of Necropsy

MADELINE A. RAE, BS, DVM, MS, Dipl ABVP-Avian



Gross necropsy and postmortem diagnostic testing are important parts of avian medicine, requiring a systematic approach to the examination of organs and the collection of samples.

Necropsy examination functions as more than a way to satisfy the curiosity of the client, breeder or attending veterinarian — it provides important information that can be used in the diagnosis and treatment of future cases. Clinical signs and clinical pathologic findings are often not definitively explained until necropsy.

Postmortem information can be invaluable in educating clients regarding the seriousness of husbandry, nutritional and infectious disease conditions, thereby preventing them from making the same mistakes with subsequent birds. For grieving owners, necropsy findings can relieve them of some or all of the guilt associated with the death of a beloved pet.

Necropsy findings are an integral part of the flock database from which husbandry, management, treatment, vaccination and quarantine recommendations can be made.

In situations where the client may be dissatisfied with treatment or outcomes, it is wise to have a veterinary pathologist perform the necropsy. Developing a relationship with a veterinary pathologist is also highly recommended so that appropriate samples are submitted, optimizing the chances of arriving at a diagnosis. The veterinary pathologist should have training, experience and interest in companion and free-ranging birds.

Preparing for the Necropsy

The supplies needed for an avian necropsy are listed in [Table 26.1](#). Although bottles or jars of neutral buffered 10% formalin should be available for the collection of specimens for histopathology, some cautions are in order. Ensure that formalin fumes do not contact tissues that are to be cultured for bacteria or viruses, as this can compromise the culture accuracy. Make certain that formalin fumes do not come in contact with blood or tissue cytological smears, as this can severely distort staining and interpretation.

A standardized necropsy form should be used and diagnostic specimen accession forms and instructions should be readily located. All specimen containers should be clearly labeled with the appropriate information. A refrigerator, a freezer, insulated shipping containers and coolant packs should be available for appropriate handling and shipping of specimens. After the necropsy has been performed, the carcass can be frozen and saved for a period of time, as frozen tissues may be useful if initial testing is inconclusive. Have arrangements in place for appropriate disposal of carcasses and infectious wastes.

Table 26.1 | Supplies Needed for Avian Necropsy

- Gram scale
- Apron
- Mask
- Gloves
- Disinfectant
- Necropsy checklist
- Scalpel handle and blades
- Scissors*
- Thumb forceps*
- Small rongeurs or toenail nippers for brain removal and bone cutting
- Ophthalmic scissors and forceps for small passerines, neonate and dead-in-shell
- Sturdy paper plates and/or plastic cutting board
- Microscope slides and coverslips
- Blood and fluid collection tubes
- Sterile saline
- Sterile culturettes (aerobic and anaerobic)
- Sterile cotton-tipped applicators
- Magnifying head loupe
- Good light source
- Sterile sealable plastic bags or sterile plastic vials for sample collection
- Plastic screw-top jars containing 10% neutral buffered formalin
- Sterile syringes and needles
- Stains (Gram's, Wright's, Giemsa or Diff-Quik, acid-fast)
- Butane lighter or other heat source to heat-fix smears for acid-fast staining

*Autoclave one set of scissors and forceps for collecting samples aseptically.

METHOD OF EUTHANASIA

In some instances, it may be appropriate to euthanize a sick bird in order to diagnose a flock problem. The method of euthanasia is important to consider because many injectable agents can cause severe artifacts in tissues. If at all possible, barbiturates should not be injected into the coelomic cavity, thoracic cavity or heart. The barbiturates are very acidic and crystals readily precipitate, causing severe tissue destruction that may obscure gross and histologic lesions. Even intravenous barbiturate solutions can cause intravascular erythrocyte lysis and some tissue damage as the solution pools within blood-filled organs.

Gas anesthetic agents seem to provide the least amount of tissue artifact (less muscle contraction artifact, no cell lysis). Once the gas agents anesthetize the bird, there is the opportunity to collect antemortem blood samples for hematology, serum chemistries, hemoparasite detection, serology and toxicology. The gas anesthetic agents can then be followed with intravenous euthanasia agents, if needed, and the amount of injectable agent necessary is usually quite reduced.

PREPARING THE BODY

The necropsy should be performed as soon after death as is possible. In order to prevent dry feathers from insulating the body and delaying cooling, wet the feathers with a detergent and water solution. The detergent and water also decreases the dispersal of feather and fecal dust into the local environment, thereby decreasing the transmission of infectious agents.

The body should be refrigerated, not frozen. Freezing can create artifacts in the tissues that may seriously obscure histologic lesions. Postmortem autolysis also can obscure histologic lesions, so if necropsy cannot be performed within 3 days, the body should be frozen, realizing that histopathology is likely to be compromised.

When shipping a cooled body, be sure the ice packs (or other frozen coolants) do not directly touch the body as this may freeze the body tissues, especially in very small birds. Wrap the ice packs in bubble wrap or newspaper to prevent freeze damage.

GUIDELINES FOR OBTAINING THE HISTORY

A detailed history should be obtained, just as the clinician would do upon seeing a live bird for the first time in the examination room ([Table 26.2](#)) (see also Chapter 6, Maximizing Information from the Physical Examination).

Table 26.2 | Considerations for Obtaining History Information

- Start with the age of the bird and sex, if known. Breeders are often very interested in having the sex of the dead bird confirmed at necropsy. If the dead bird is from a pair that is known to have produced fertile eggs, the knowledge of the mate can be deduced from the confirmation of the dead bird's sex. The breeder then knows which sex of bird to replace in the pair.
- Determine what the bird's purpose is, whether it is a dear pet, a display bird or a breeder bird.
- It is important to obtain a detailed description of the diet fed, and for how long, including any supplements and grit, brands used and their storage. Keeping a stored sample frozen in an airtight container may be useful. Quality control by the manufacturer and storage of formulated diets can be a problem. The resulting products may be over-formulated or rancid, resulting in toxic levels over time.
- Determine if there have been changes in appetite and/or droppings.
- How is water provided and how often is it changed?
- Have feeding practices or the diet changed recently?
- If this is a chick that is being hand-fed, obtain a detailed description of the feeding practices, brand of food with amounts and times per day, as well as any charts documenting weight loss or gain.
- Is the bird allowed to fly freely within the house?
- Ask for a description of the cage and its placement within the house (eg, near the kitchen where exposure to toxic fumes may occur).
- Is bedding material used and how often is the cage cleaned? What products are used for cleaning?
- Are there toys in the cage and does or can the bird chew on them?
- If this bird is from an aviary, a description of the aviary and floor plan can be very helpful.
- How many other birds are in the home or aviary and how many have died in the last year? Are any other birds in the household or aviary ill? Was the deceased bird in quarantine? Are any humans in contact with the ill birds?
- Have there been any recent changes in environmental conditions such as temperature changes?
- Have there been recent additions to the aviary or household?
- Ask about the reproductive history of the bird, such as a recent history of egg laying, dystocia or feeding of chicks.
- Obtain a description of recent clinical signs noticed prior to death and their duration.
- Ask about any other previous illnesses or conditions. Were any medications or treatments given to the bird? Be sure to ask about prescription medications as well as over-the-counter preparations.

PREVENTING CONTAMINATION

Perform the necropsy in a well-lighted, well-ventilated area (preferably under a fume hood), and wear gloves, a mask and, if possible, a disposable apron. Aerosols from feathers, feces and exudates can be infectious. This is particularly important with cases of chlamydophilosis and mycobacteriosis, which can be zoonotic. However, it also is important to contain the feather dander and feces in cases of avian polyomavirus and psittacine circovirus infections, so as not to contaminate the premises, your clothing or other adjacent birds.

Disinfectant solutions should be readily available for clean-up after the necropsy, but neither these solutions nor their fumes should come in contact with tissues being collected, as they may lyse cells and destroy microorganisms needed for culture.

Step-by-Step Necropsy Procedure

The particular routine used for gross necropsy of birds can vary, but what remains the same is that all organs and systems are examined, and the use of a checklist will ensure this. Use the Necropsy Checklist (Table 26.3) to document all findings, both normal and abnormal. Make this checklist a part of the medical record and send a copy to the veterinary pathologist with any fixed tissues.

It is important to collect samples of everything (all organs, the grossly normal and abnormal). Labeling sealable bags and formalin jars prior to the necropsy with the owner's name and the tissues enclosed can save time and prevent interruptions in the flow of the necropsy.

After the necropsy is completed, the decision of which samples to send and what tests to request can be made, and at the very least, the diagnosis will not be cremated with the carcass.

EXTERNAL EXAMINATION

The necropsy begins with an external examination. Record the band number and scan for microchips; these can be removed, labeled and saved as proof of identification. Weigh the bird using a gram scale and record the weight on the checklist. Palpate for obvious fractures; radiographs may be warranted in some instances.

Examine the skin and feathers: often, feather abnormalities may not be visible while the feather remains in the follicle. For example, the concentric pinching of the feather shaft, seen in psittacine circovirus infection, may not be visualized until the feather is plucked from the follicle. Look for stress bars in the wing and tail primaries (Fig 26.1). Collect multiple blood feathers, both plucked and in the follicle, along with any skin lesions (Fig 26.2) and place them in formalin. Check for any signs of

Table 26.3 | Necropsy Checklist

Owner's Name	Date of Necropsy
Animal's Name	Date of Death
Species	Euthanasia Method
Age	Sex
Band/Microchip #	Tattoo?

Organ(s)	Normal	Abnormal	Description
Skin/Feathers			
Beak/Oral Cavity/Tongue			
Eyes/Ears/Conjunctivae			
Sinuses/Choana/Nasal Cavity			
Skeletal muscle/Bones/Joints			
Liver/Gall Bladder, if present			
Spleen			
Thyroids/Parathyroids			
Trachea/Lungs/Airsacs			
Kidneys/Adrenals			
Testes/Ovary/Oviduct			
Crop/Esophagus			
Proventriculus/Ventriculus			
Duodenum/Pancreas			
Jejunum/Ileum/Ceca, if present			
Colon/Cloaca			
Bursa/Thymus			
Brain/Meninges			
Spinal Cord/Vertebrae/Nerves			
Bone/bone marrow			
Middle & inner ear			
Heart/Great Vessels			
Gut contents wet mount results:			
Gut contents dried smear results:			
Organ impression smear results:			
Tissues in formalin:			
Tissues frozen:			

trauma or bruising. In neonates, closely examine the umbilicus for cleanliness and the adequacy of healing.

Examine the unfeathered portions of the legs and the feet for poxvirus lesions, bumblefoot, herpesvirus pododermatitis and self-mutilation (Figs 26.3, 26.4). Examine the uropygial gland, found at the base of the tail in some species, and collect it for histopathologic evaluation, as this can be a site of chronic inflammation and neoplasia.

Evaluate the beak, both the external and the intraoral surfaces (Fig 26.5). Open the mouth. Look at and under the tongue for abnormalities. Look in the choanal slit for mucus and exudate and for blunting of the choanal papillae (Fig 26.6). Salivary gland enlargement can occur at the base of the tongue and can be due to hypovitaminosis A, bacterial abscesses or, rarely, mycobacterial infections.

The nares and ear canals should be clear and free of

debris or exudate. Examine the conjunctivae and the nictitating membranes. In Columbiformes, these tissues can be collected for *Chlamydophila* diagnostics, as they may contain elementary bodies.

The infraorbital sinuses should be opened as aseptically as possible, and swabs or aspirates collected for cytology and culture of bacteria, *Mycoplasma* and fungi (Figs 26.7, 26.8). Bacterial sinusitis is quite common in psittacines, but also occurs in passerine species, and caseous exudate is often seen (Fig 26.9).

In cockatiels (*Nymphicus hollandicus*) with “lockjaw,” sinusitis and temporomandibulitis are common, as well as myositis of the mandibular muscles. The mandible and its attached muscles can be placed in formalin for histopathology. In these cases, bacteria such as *Bordetella avium*, *Enterococcus*, *Escherichia coli* and *Enterobacter* may be isolated. It is important to indicate to the



Fig 26.1 | Dystrophic feathers from a sulfur-crested cockatoo (*Cacatua galerita*) infected with psittacine circovirus. Note the concentric pinching of the feather shafts.



Fig 26.3 | Cutaneous pox lesions of the feet in a red-tailed hawk (*Buteo jamaicensis*).



Fig 26.4 | Avascular necrosis of the distal toes of a black-crowned night heron (*Nycticorax nycticorax*).

bacteriology laboratory that *B. avium* is suspected because this organism is somewhat fastidious and colonies may take longer to appear. *Bordetella avium* also may cause tracheitis, bronchitis and pneumonia in cockatiels and rarely in other psittacines.

Several *Mycoplasma* species have been implicated in conjunctivitis and sinusitis in psittacines and passerines, but these require special media for isolation and are recovered uncommonly. In small passerines, cross-sections of the nasal cavity and sinuses can be submitted



Fig 26.2 | Dermal lymphosarcoma in a western screech owl (*Otus kennicottii*).



Fig 26.5 | Cutaneous pox involving the commissures of the mouth in a red-tailed hawk.

in formalin, decalcified if needed, and examined histologically. Sometimes the nasal cavity epithelium may be the only site of viral inclusions diagnostic for canarypox. Cryptosporidial rhinitis and conjunctivitis also can be diagnosed with this method.

COELOMIC CAVITY

The coelomic cavity examination is begun by placing the body in dorsal recumbency, incising the skin of the abdomen and peeling it back caudally over the abdomen

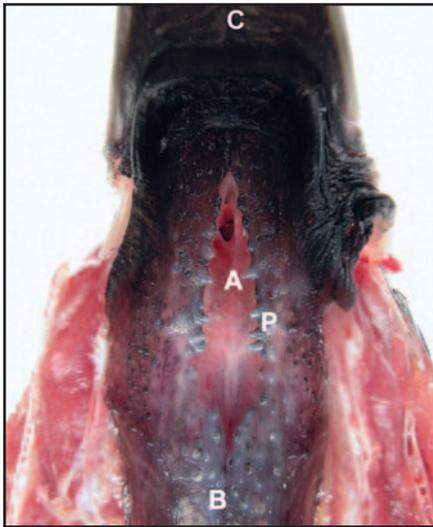


Fig 26.6 | Normal choanal slit of a blue-fronted Amazon parrot (*Amazona aestiva*). Choanal slit (A), caudal palate containing salivary glands (B), choanal papillae (P), palatine beak (C).

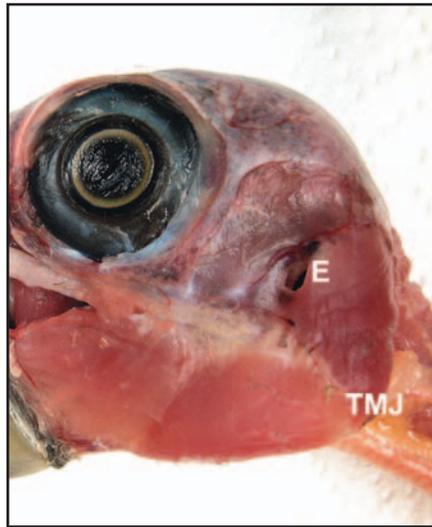


Fig 26.7 | View of the avian skull with the lateral wall of the infraorbital sinuses cut away in a blue-fronted Amazon parrot. Exudate may accumulate in the temporo-mandibular joint (TMJ), especially in cockatiels. Ear (E).

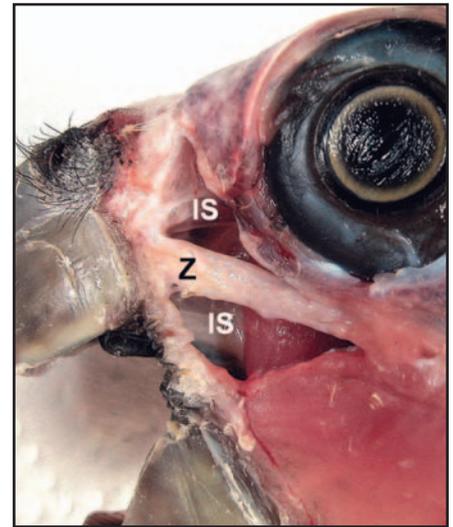


Fig 26.8 | View of the infraorbital sinuses in a blue-fronted Amazon parrot with the lateral wall removed. Infraorbital sinuses (IS), zygomatic or jugal arch (Z).

and cranially over the pectoral muscle mass. Assess the condition of the pectoral muscle, as this is a good measure of weight loss (Fig 26.10). Notice whether subcutaneous fat is present or absent and whether there is any bruising or edema. Grasp the sternum with thumb forceps and slightly elevate, maintaining tension on the abdominal wall. Make a transverse incision with the scalpel blade just caudal to the edge of sternum, being careful not to lacerate the liver. Remove the keel and pectoral muscles in one piece by cutting through the ribs and shoulder girdle with scissors or rongeurs.

Liver and Spleen

Assess the size, color and consistency of the liver. Hepatic margins should be sharp and not extend beyond the caudal edge of the keel in an adult bird (Figs 26.11-26.13). Note whether a gall bladder is present or absent, as not all species have one. Assess the condition of the air sacs and coelomic surfaces that are visible. If coelomic fluid is present, collect it with a sterile syringe for analysis. Aseptically collect samples of air sacs next, since they are delicate structures that readily disappear with further manipulation of the organs. Aseptically collect liver samples: one sample each for bacteriology, virus isolation or DNA probe testing, *Chlamydophila* testing and histopathology, and use any remaining tissue for toxicology and/or impression smears.

Grasp the ventriculus, elevate and incise through the attached membrane/air sac along the left margin and rotate the ventriculus counterclockwise to find the spleen. The spleen is nestled in the curve between the proven-

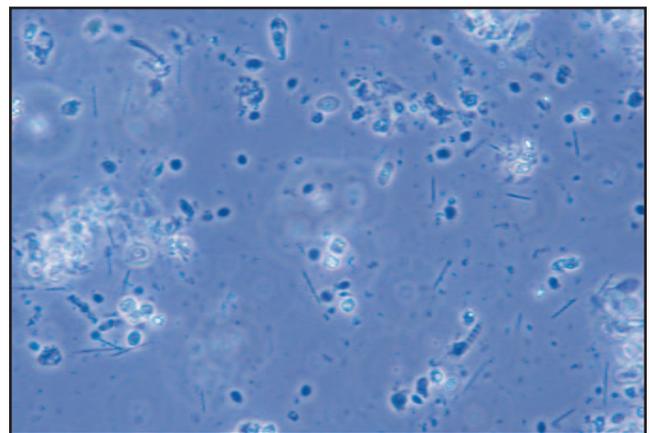


Fig 26.9 | Spirochetes in a wet mount of exudate from the infraorbital sinuses of a cockatiel chick (*Nymphicus hollandicus*), photographed under phase contrast.

tricus and the ventriculus (Fig 26.14). Evaluate the size and shape of the spleen. Determining whether it is of normal size for the bird being necropsied requires some practice, so measuring the diameter can be helpful. The spleen is round in some species, such as Psittaciformes and Galliformes, and elongated or sock-like in Passeriformes and Columbiformes. Note the color and the presence of any pale foci in the spleen. Collect the entire spleen, dividing it into three samples: one for virology, one for *Chlamydophila* diagnostics and one for histopathology. The spleen is the single most important sample for the histopathologic diagnosis of avian polyomavirus infection, since this is where viral inclusions are most abundant.

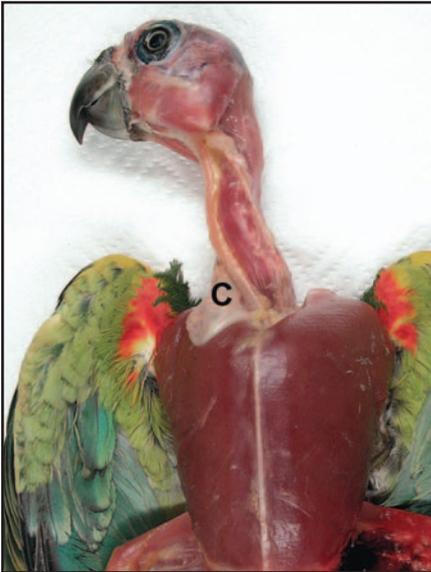


Fig 26.10 | View of the normal pectoral muscles of a blue-fronted Amazon parrot following removal of the skin. Crop (C).

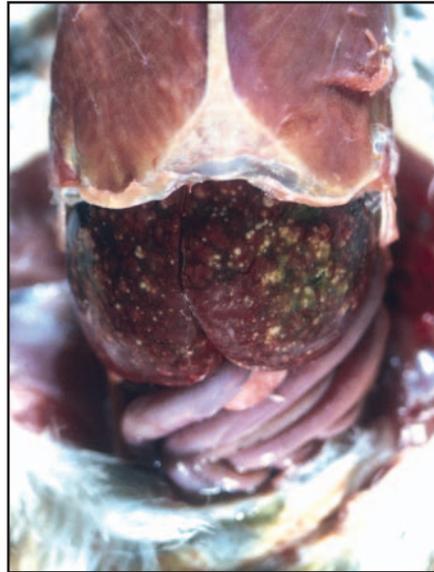


Fig 26.11 | The liver extends beyond the caudal edge of the keel, indicating hepatomegaly in this mature red-tailed hawk with multiple mycobacterial granulomas in the liver.

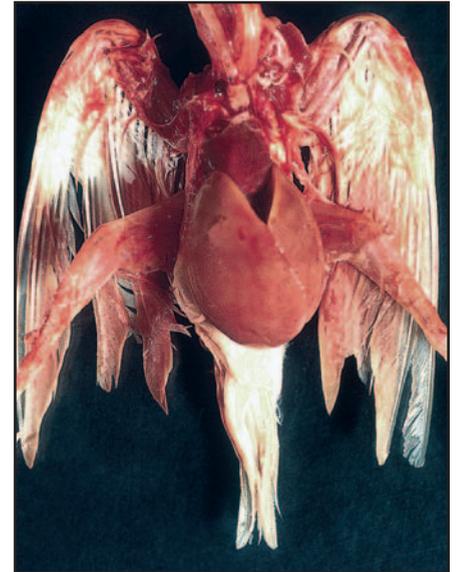


Fig 26.12 | Marked hepatomegaly in a Lady Gouldian finch (*Chloebia gouldiae*). In this case, the hepatomegaly was due to lymphosarcoma, but a number of diseases can cause marked hepatomegaly in passerines.

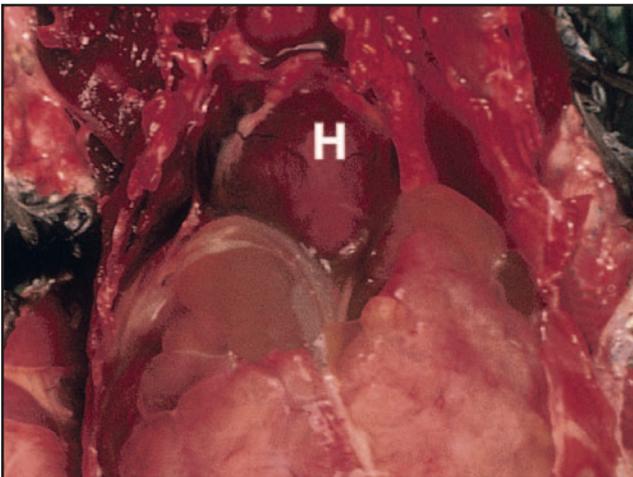


Fig 26.13 | Cholangiocarcinoma in an Amazon parrot (*Amazona* sp.). This tumor can have a very pleomorphic gross appearance. Heart (H).

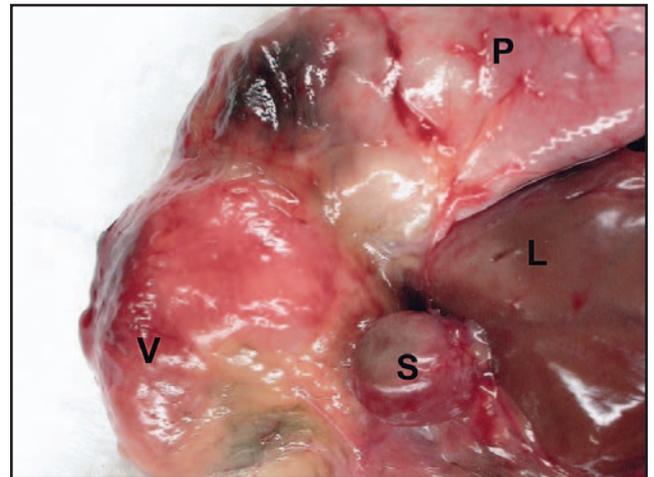


Fig 26.14 | Normal spleen in a blue-fronted Amazon parrot. Spleen (S), liver (L), proventriculus (P), ventriculus (V).

Genitourinary

Reflect the ventriculus and the intestinal tract to the right side of the bird to view the adrenals, gonads and kidneys, leaving the unopened gastrointestinal tract for last to avoid contamination of the other abdominal organs. The adrenals are often obscured by active gonadal tissue, so it is easier to collect the cranial division of the kidney with the adrenal and gonad(s) attached for histopathology. Adrenitis is sometimes noted in unexplained death and may be the only abnormality in some psittacines with proventricular dilatation disease (PDD).

Sex the bird visually. In most species, only the left ovary and oviduct develop in females, but both testes develop in male birds. The gonads may be pigmented (brown or

black) in some species, most notably cockatoos. Note the degree of development of the ovary and oviduct. Is there follicular development? If so, record the general size of the follicles. Is the oviduct hypertrophied?

Open the oviduct to look for exudate and tumors, and collect samples for bacteriology and histopathology as needed. Neoplasms of the oviduct may occur in the mucosa or myometrium. Testicular tumors are common in budgerigars (*Melopsittacus undulatus*) and ducks (Fig 26.15), but seasonal testicular enlargement also occurs. In some species, such as in many passerines, this enlargement can be mistaken for neoplasia; histopathology can usually distinguish between these two changes.

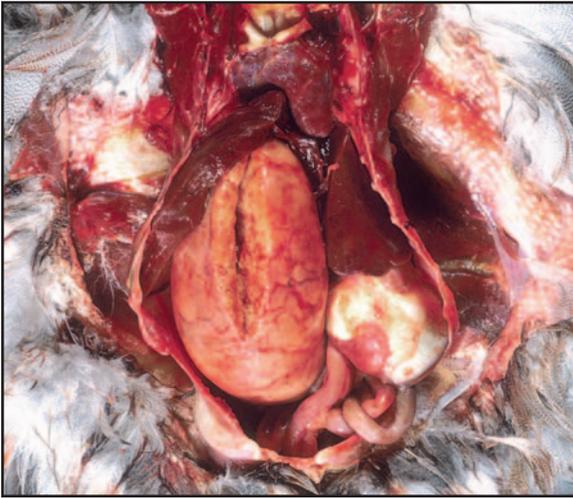


Fig 26.15 | Seminoma of the testes in a mallard duck (*Anas platyrhynchos*).

The kidneys are nestled in the renal fossae of the synsacrum, with the lumbosacral plexus lying deep to the caudal divisions of the kidney bilaterally (Fig 26.16). The ureters run down the ventral surface of the kidneys. In addition to the kidney/adrenal/gonad tissue collected as described above for histopathology, aseptically collect additional renal samples for virology, toxicology and bacteriology (if exudate is present).

In small birds (under 30 g), one can make an en bloc excision of the kidneys still in situ within the synsacrum and place this in formalin. After fixation, renal dissection is easier and/or the synsacrum can be decalcified and cross-sections of kidney together with bone can be cut. After removal of the kidneys, evaluate the lumbosacral plexus, especially in cases of pelvic limb weakness or malfunction. Samples of these nerves can be collected in formalin for histopathologic evaluation.

THORACIC INLET

Move to the thoracic inlet region. Identify the thyroids and parathyroids, located cranial to the heart and adjacent to the carotid arteries bilaterally, and collect them for histopathology (Fig 26.17). Goitrous changes were once quite common in budgerigars, but are less so with the advent of commercial diets. Hyperplastic goiter has been reported in juvenile macaws recently, but the cause is currently unknown. Lymphocytic thyroiditis also may be seen histologically, especially in Amazon parrots (*Amazona* spp.). Thyroid tumors are uncommon, but seem to be observed most often in cockatiels, where they tend to be very vascular.

Normal parathyroids are barely visible. When they are prominent, metabolic bone disease should be consid-

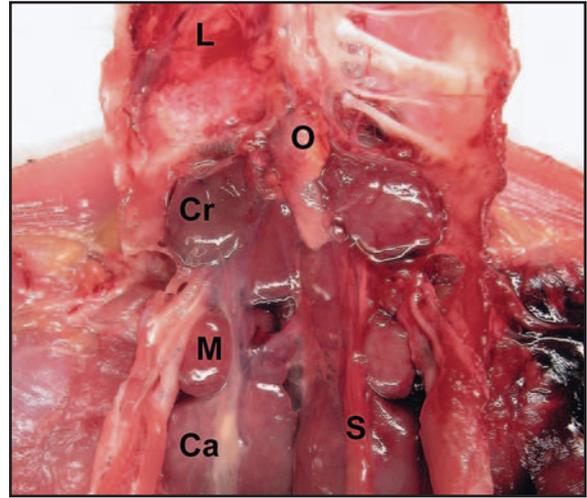


Fig 26.16 | Dissection displaying kidneys, reproductive structures and lung from a normal blue-fronted Amazon parrot. Lung (L), ovary, immature (O), cranial division (Cr), middle division (M) caudal division (Ca) of the kidney, salpinx or oviduct (S).

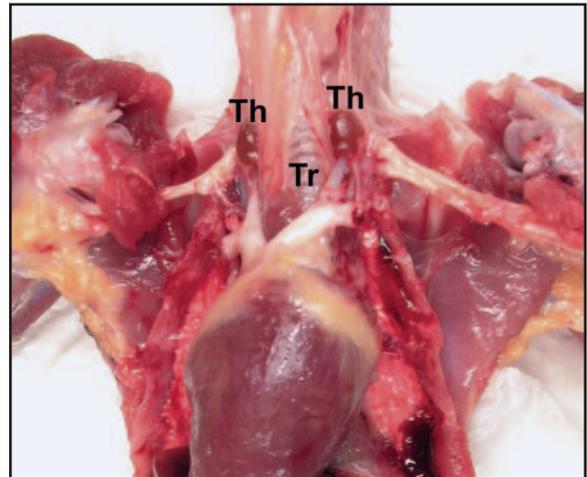


Fig 26.17 | View of the thoracic inlet, thorax and cranial abdomen following removal of the keel and pectoral muscles of a blue-fronted Amazon parrot. Thyroids (Th) are located cranial to the heart on either side of the trachea (Tr), close to the carotid arteries. The parathyroids are located at the caudal margin of the thyroids and are barely visible in normal birds.

ered. Parathyroid hypertrophy is usually the only gross pathologic lesion found in the hypocalcemia syndrome of African grey parrots (*Psittacus erithacus*).

In young birds, multiple pale lobules of thymic tissue can be found along the cervical region, from the jaw to the thoracic inlet (Figs 26.18, 26.19). Thymic tissue can be collected for virology and histopathology. Thymomas occasionally are seen as circumscribed, fairly encapsulated masses; they tend to be slow growing, undergoing cystic degeneration with large areas of hemorrhage. Thymic tissue is usually quite difficult to detect in adult birds.

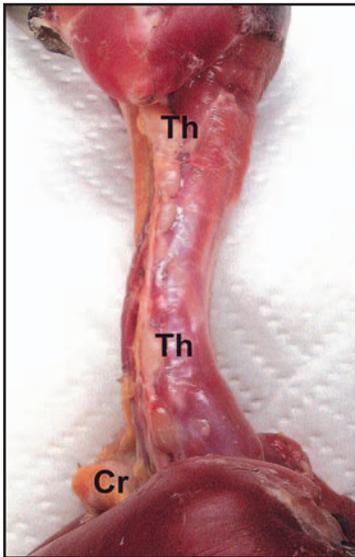


Fig 26.18 | Left lateral view of the neck of a normal young blue-fronted Amazon parrot revealing multiple lobules of thymic tissue along its entire length from the jaw to the thoracic inlet. Thymus (Th), crop (Cr).

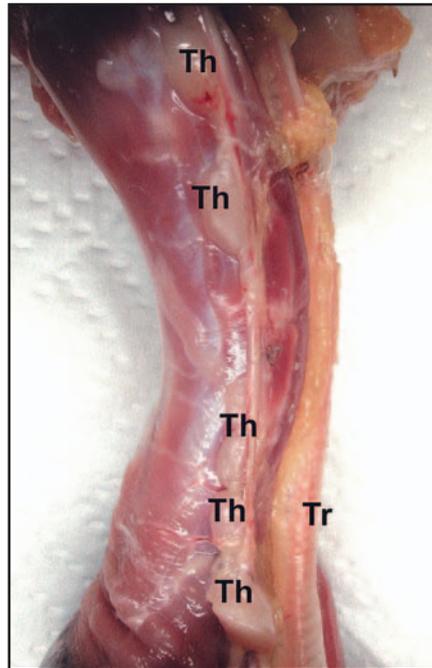


Fig 26.19 | Right lateral view of the neck of a young blue-fronted Amazon parrot revealing multiple lobules of thymic tissue along its entire length from the jaw to the thoracic inlet. Thymus (Th), trachea (Tr).

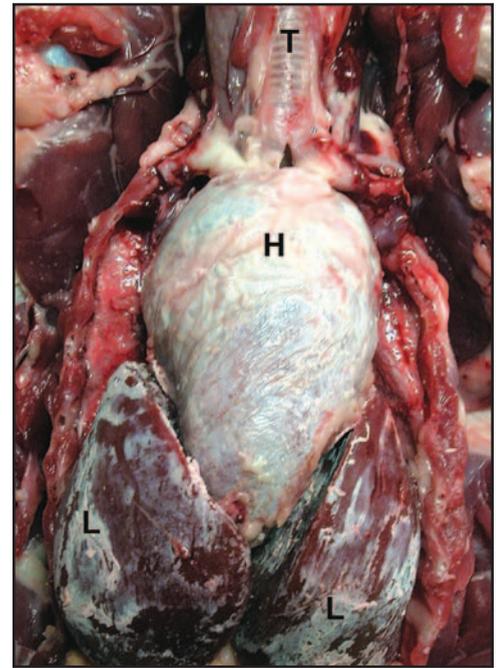


Fig 26.20 | Visceral gout in a red-tailed hawk with severe deposition of urates in the pericardial sac and in the perihepatic membranes. This is a very important gross lesion for the practitioner to recognize, because the urates may dissolve out of the tissue when placed in formalin. A smear of the white material can be examined under polarized light to reveal the typical urate crystal structure. Trachea (T), liver (L), heart (H).

Cardiac

Examine the heart, pericardium and great vessels.

Visceral gout can cause the deposition of white, mucoid urate material in the pericardial sac (**Fig 26.20**). View a smear of this material under polarized light to confirm the presence of uric acid crystals. It is very important to recognize the gross appearance of visceral gout, because formalin fixation may dissolve the uric acid crystals and they may not be visible on histopathology (crystals do not dissolve if fixed in ethanol, but this is usually not a practical fixative for other reasons).

Suppurative pericarditis can be caused by a variety of bacteria such as *Pasteurella* and *Chlamydoiphila*.

Cytologic examination of the pericardial exudate may reveal the causative organisms. Portions of the pericardial sac can be included in the tissues used for *Chlamydoiphila* diagnostics. Hydropericardium is a common finding in avian polyomavirus infection in juvenile psittacines.

Prior to removing the heart from the thoracic cavity, heart blood can be collected using a sterile syringe and needle for bacteriology. Smears of heart blood can be stained with Wright's stain and examined for hemoprotozoa and microfilaria, or Gram's stained to look for bacteria.

Cardiomyopathy, usually the dilative form, can occur in birds. The cause of the cardiomyopathy is often obscure by the time it becomes a clinical problem, but myocardial degeneration and fibrosis are often seen histologically. After removing the heart and great vessels, open the heart in the direction of blood flow, using water to rinse away blood and clots. (See Chapter 12, Evaluating and Treating the Cardiovascular System for measurements of the heart). Look for thrombi, valvular endocarditis lesions and pale areas in the myocardium. Congenital cardiac anomalies are rarely diagnosed.

Open the great vessels to look for atherosclerosis, which may involve the aorta, pulmonary artery or carotids. Atherosclerosis is characterized grossly by yellowish, raised, intimal plaques, but occasionally may be so severe that the carotids are completely obstructed. Mineralization of the great vessels also may occur in association with atherosclerosis or may be related to renal disease and hypervitaminosis D.

Atherosclerosis is most commonly seen in African grey parrots, where it can be mild to moderate, but also in obese, older Amazon parrots, older macaw species (*Ara* spp.) and captive raptors, where it can be so severe that it results in acute death (**Fig 26.21**). Atherosclerotic

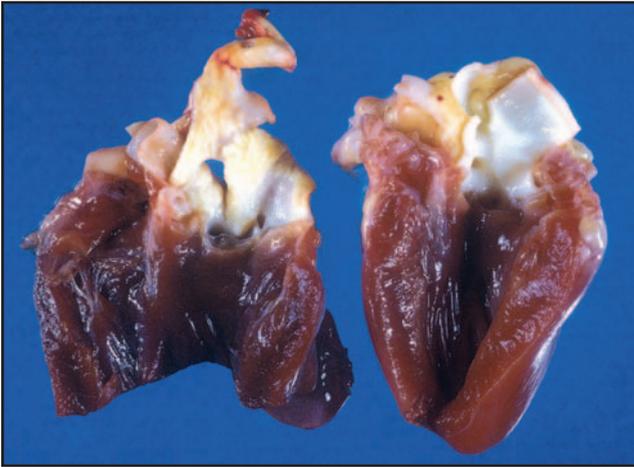


Fig 26.21 | Atherosclerotic plaques on the luminal surfaces of the great vessels of the heart in an Amazon parrot (*Amazona* sp.). Note the smooth, glistening luminal surfaces of the normal heart on the left.

lesions also may be found in the coronary arteries, but usually these lesions are discovered upon histopathologic examination. Atherosclerosis is a commonly missed diagnosis because the vessels are not opened and examined.

It is best to place most of the heart in formalin so that multiple sections can be cut for histopathologic evaluation. Pale foci or streaks can indicate degenerative myopathy related to vitamin E/selenium deficiency, or myocarditis associated with septicemia or viral diseases such as West Nile virus or PDD. Petechial and ecchymotic epicardial hemorrhages are commonly seen in cases of acute death from avian polyomavirus.

Lungs

Examine the lungs in situ prior to removing them. Avian lungs are fixed in place within the avian thoracic cavity and are not freely moveable. Removal requires gentle teasing of the lung tissue away from the ribs. The avian lung is one tissue in which gross lesions may appear quite significant, but upon histopathologic evaluation turn out to be just passive congestion. Conversely, grossly normal lungs may contain significant histologic lesions. So, it is wise to always include lung for histopathology, even if it appears grossly normal. Collect a portion of the lung for bacteriology and virology and place the rest in formalin for histopathology. Because lesions can be focal or multifocal, it is best to include a large portion of at least one lung for histopathology.

In canaries and mynahs, a Wright's-stained impression smear of lung (along with impressions from liver and spleen) is very important in detecting the monocytic form of toxoplasmosis, as *Toxoplasma* organisms are not usually visible on histopathology. *Sarcocystis* organisms, bacteria and fungi also can be seen in impression smears of the lung.

ORAL CAVITY AND GASTROINTESTINAL TRACT

Cutting through the mandible at one of the lateral commissures allows access to the caudal pharynx and visualization of the glottis (Figs 26.22, 26.23). The larynx and trachea can then be opened down to the tracheal bifurcation, looking for hemorrhage, exudate, foreign bodies, granulomas, and parasites such as respiratory mites or *Syngamus* nematodes. Laryngeal papillomatosis can occur in the pharynx and may occlude the glottis (Fig 26.24).

Tissue samples should be collected for histopathology and virus isolation, as well as bacteriology and fungal culture if warranted. Fungal tracheitis, especially at the syrinx, can be diagnosed by cytologic examination of exudate or granulomatous material, fungal culture and/or by histopathology. Rarely, mycobacterial organisms can cause syringeal granulomas.

In canaries, *Enterococcus faecalis* can cause chronic tracheobronchitis; culture of the tracheal lumen is necessary for diagnosis. Viral tracheitis is rare in psittacine birds, but a herpesviral tracheitis, bronchitis and airsacculitis have been reported in *Neophema* parrots. Canarypox can produce a severe tracheobronchitis with intracytoplasmic inclusions. A severe, chronic tracheobronchitis can be seen with *Bordetella avium* in cockatiels; this organism also is often associated with the "lockjaw" syndrome of sinusitis and temporomandibulitis.

Esophagus and Crop

Going back to the pharynx, the cut can extend downward the length of the esophagus and into the crop, looking for lacerations, punctures, peri-esophageal abscesses and other abnormalities. In game birds, the esophagus and crop may exhibit moderate to marked thickening and mucus production due to capillariasis. A wet mount scraping from the crop can reveal the typical bipolar capillariid ova. In juvenile psittacines, thickening and "Turkish towel" appearance of the crop mucosa is often due to candidiasis, and either a wet mount smear, cytology or Gram's stain of a crop mucosal scraping can be diagnostic (Fig 26.25).

Trichomonads can be found in wet mounts from the oral cavity and/or crop of Columbiformes and raptors, but also may occasionally be found in the crops of budgerigars and passerines. The crop contents can be collected in a plastic bag and frozen, if there is any suggestion of toxin ingestion. A large section of crop, to include a large vessel and adjacent nerve, should be collected for histopathology, since PDD lesions are often closely associated with the nerves.



Fig 26.22 | Cutting through the mandible allows opening of the entire oral cavity and better visualization in a blue-fronted Amazon parrot.

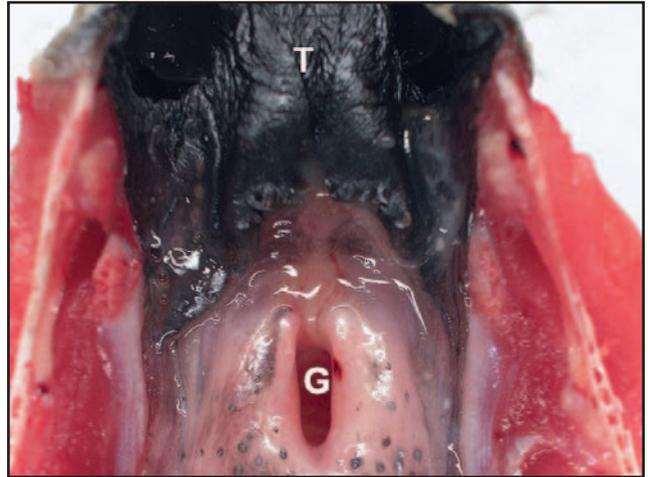


Fig 26.23 | Normal glottis from a blue-fronted Amazon parrot. Tongue (T), glottis (G).



Fig 26.24 | The glottis of this lilac-crowned Amazon parrot (*Amazona finschi*) is occluded by laryngeal papillomatosis.



Fig 26.25 | Severe crop mycosis in a cockatiel chick.

At this point, the esophagus distal to the crop can be transected. Caudal traction of the distal esophagus and sharp dissection of the mesenteric attachments can be utilized to remove the entire gastrointestinal tract.

Bursa of Fabricius

Continue the dissection to make a circular incision around the vent, leaving a margin of intact vent skin and the bursa of Fabricius attached to the tract. The bursa is present in young birds usually less than 6 to 12 months of age and is located dorsal to the cloaca. The bursa should always be collected when it is present and divided in half. Submit one half in formalin for histopathology and save the other half for virology and/or DNA probe testing.

The bursa is important in diagnosing psittacine circovirus, especially in young African grey parrots that die acutely without feather lesions, since the bursa may be the only site where viral inclusions are found. Circoviral inclusions also can be found in the bursa of young pigeon squabs dying from a variety of secondary infections. Lesions in the bursa are often non-specific as to etiology, but can indicate the acuteness or chronicity of stress.

Proventriculus and Ventriculus

Open the distal esophagus with scissors, continuing on into the proventriculus and ventriculus. Evaluate the stomach contents for amount and any foreign material. Washing the contents into a bowl or strainer can allow the food material to be rinsed away, leaving metallic and other foreign bodies behind. Collect and freeze the contents for possible toxicologic analysis. Rinse the mucosa with water and make wet mount and dried smears of mucus and/or mucosal scrapings. Do not separate the proventriculus and ventriculus.

The isthmus (the junction between the proventriculus and ventriculus) is a common site for avian gastric yeast (formerly known as megabacteria); its suggested new name is *Macrorhabdus ornithogaster* (see Chapter 30, Implications of *Macrorhabdus* in Clinical Disorders) (Fig 26.26). Grey-cheeked parakeets (*Brotogeris pyrrhopterus*) seem to be prone to the development of gastric carcinoma at the isthmus and the gross lesions are often unexciting. Collect a large specimen of proventriculus, isthmus and ventriculus (all in one piece if possible), containing at least one large serosal nerve and blood vessel, for histopathology.

In small birds, the entire proventriculus and ventriculus can be placed in formalin. A large specimen

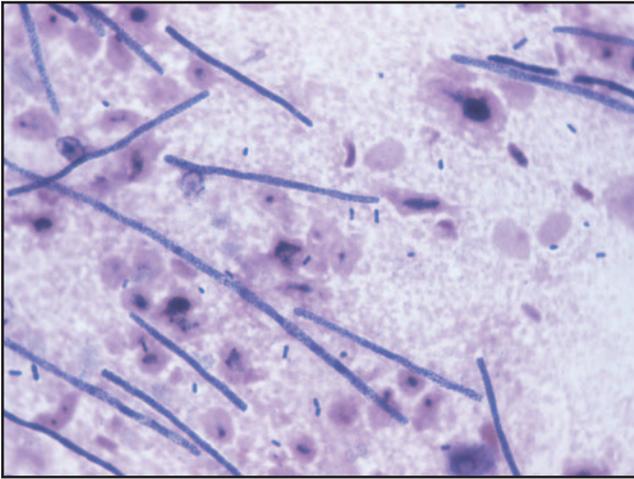


Fig 26.26 | Large gram-positive organisms consistent with the yeast *Macrorhabdus ornithogaster* (formerly known as megabacteria) are seen in a Gram's-stained smear of intestinal contents from a cockatiel. Note the size difference between these fungal organisms and the smaller gram-positive bacilli.

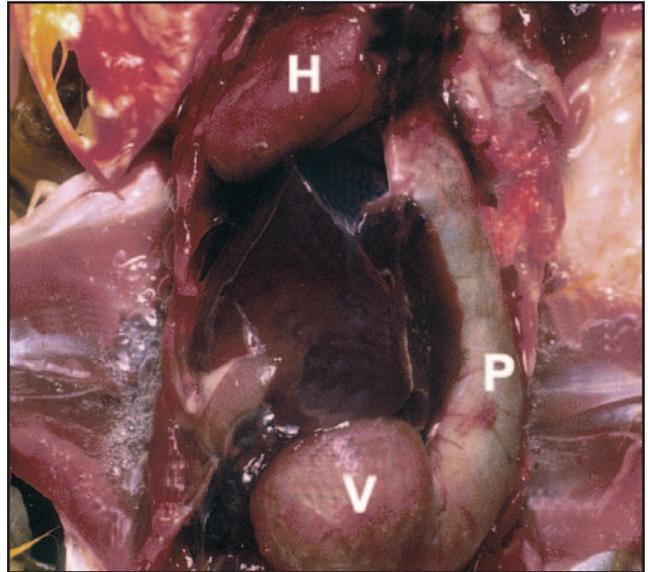


Fig 26.27 | Marked dilatation of the proventriculus (P) in a macaw (*Ara ararauna*) with proventricular dilatation disease (PDD). H= heart, V= ventriculus.

allows multiple sections to be examined by the veterinary pathologist in the search for nerves and plexi. Dilatation of the proventriculus and/or ventriculus is a hallmark gross lesion of PDD, but in juvenile psittacines being hand-fed, these organs also may be dilated as a normal finding. Histopathology is required to differentiate between PDD and normal juvenile underdevelopment of the proventriculus and ventriculus (Fig 26.27).

Foreign body penetration of the ventricular wall can occur in any species, but is most common in waterfowl and ratites. Nutritional muscular dystrophy (degenerative myopathy) can be seen in some species as white streaks in the ventricular muscle as a manifestation of vitamin E/selenium deficiency. Endoventricular mycosis (fungal invasion of the koilin lining of the ventriculus) can be seen histologically and is a common finding in debilitated passerines, despite the usually unremarkable gross appearance.

Duodenum and Pancreas

Open the outflow tract from the ventriculus and proceed into the duodenal loop. The largest limb of the pancreas lies in the duodenal loop mesentery while the small splenic lobe of the pancreas is located adjacent to the spleen. Pancreatic lesions are fairly common histologically, but gross lesions may not be very striking. The pancreas also is one of the first organs to undergo post-mortem autolysis.

Quaker parrots (*Myiopsitta monachus*) are prone to the development of acute pancreatic necrosis of unknown etiology. Fat necrosis and serositis may accompany pancreatitis and pancreatic necrosis. Quaker parrots that sur-

vive the initial insult may develop severe pancreatic atrophy and fibrosis. Inclusion body pancreatitis can be seen with herpesvirus and adenovirus infections. Lymphoplasmacytic pancreatitis in *Neophema* parrots is associated with paramyxovirus infection. Pancreatic necrosis also is a common lesion in West Nile virus infection.

Vacuolar changes and necrosis of acinar cells may be seen in zinc toxicosis, but these lesions can be readily obscured by even mild postmortem autolysis. The pancreas concentrates zinc in the acinar cells and should be collected for toxicologic analysis, along with liver and kidney, to diagnose zinc toxicity. Collect a sample of pancreas for virology. Also submit in formalin a transverse section through the duodenal loop with pancreas attached, as this helps to identify the duodenum.

Yolk Sac

In neonate, the yolk sac and stalk should be evaluated for the degree of absorption. In psittacine and passerine chicks, the yolk sac is usually quite tiny by 3 days after hatching. Collect a sterile sample of the yolk material for culture and place the rest of the yolk sac (wall and contents) into formalin. Yolk sacculitis and yolk sac retention are common problems in neonatal ratites.

Intestines

Continue opening the intestine through the jejunum and ileum to the ceca (if present in the species) and colon. Collect sections of intestine for histopathology. Opened intestinal sections are usually best, as this gives the mucosa a chance to fix rapidly (Fig 26.28). Do not disturb the mucosa by scraping or handling, as artifacts



Fig 26.28 | Intestinal volvulus in an ostrich (*Struthio camelus*).

can confuse or obliterate the histologic diagnosis.

Wet mounts of intestinal contents (usually two different sites) are helpful in diagnosing parasitic and bacterial problems. Wet mounts should be examined for parasite ova and oocysts, as well as flagellates, yeast and motile bacteria. Sections of bowel can be tied off with string or suture and submitted for culture. In some cases, both aerobic and anaerobic culture may be warranted (**Fig 26.29**).

If necrotic lesions are encountered in the intestinal mucosa, clostridial disease should be considered. Quail disease caused by *Clostridium colinum* is a common problem in quail, and typical “button ulcers” can be seen in the intestines as well as “crateriform” necrotic lesions in the liver. Clostridial enteritis, usually caused by *Clostridium perfringens*, is becoming more commonly recognized in psittacines, especially nectar eaters such as lorries and lorikeets. Clostridial organisms in large numbers can cause acute necrohemorrhagic enteritis. Finding large gram-positive bacilli, with or without spore formation, as the primary organism on a Gram’s-stained smear of intestinal contents gives a presumptive diagnosis that should be followed by anaerobic culture (**Fig 26.30**). Because exposure to oxygen in the air can inhibit clostridia, it is wise to tie off a loop of unopened, affected bowel with string or suture and place it in a sealable bag with the air evacuated prior to sending it for anaerobic culture.

A wide variety of other bacteria can cause enteritis and septicemia. Gram-negative organisms, especially Entero-



Fig 26.29 | Fibrino-suppurative enteritis in a tufted puffin (*Fratercula cirrhata*).

bacteriaceae, are common infectious agents in psittacines and passerines. In addition, *Campylobacter* spp. and *Yersinia pseudotuberculosis* are more common in canaries and exotic finches. *Campylobacter* organisms usually require special media and microaerophilic incubation conditions, so it is wise to alert the bacteriology laboratory when this organism is suspected.

Multifocal granulomas or thickened areas of bowel can be indicative of mycobacteriosis. These sites should be collected for histopathology, and special acid-fast tissue stains can be applied to paraffin sections to demonstrate the organisms. Alternatively, impressions or scrapings from these sites can be stained with a rapid acid-fast stain for a quick, presumptive diagnosis. Sections of affected bowel can be collected for mycobacterial culture.

Intestinal neoplasia is fortunately uncommon in birds, but needs to be included in the differential diagnosis of thickened or proliferative bowel lesions.

Flagellate protozoa and coccidial organisms also may produce enteritis. Flagellates (including *Giardia* spp. and *Cochlosoma* spp.) are diagnosed by fresh wet mount smears of intestinal contents, but they are nearly impossible to diagnose on histopathology. Coccidiosis can be diagnosed by wet mount smears of intestinal scrapings and by histopathology. Nematode and cestode parasites are uncommon in domestically raised psittacines and passerines, but geographic pockets of these parasites may exist and should always be considered (**Fig 26.31**). These parasites are still common in ground-feeding and feral or wild birds.

Ceca

Many species of birds do not possess ceca. Psittacines do not. Passerines and Columbiformes have tiny vestigial ceca composed of lymphoid tissue, while Galliformes, Anseriformes and ratites possess large bilateral ceca.

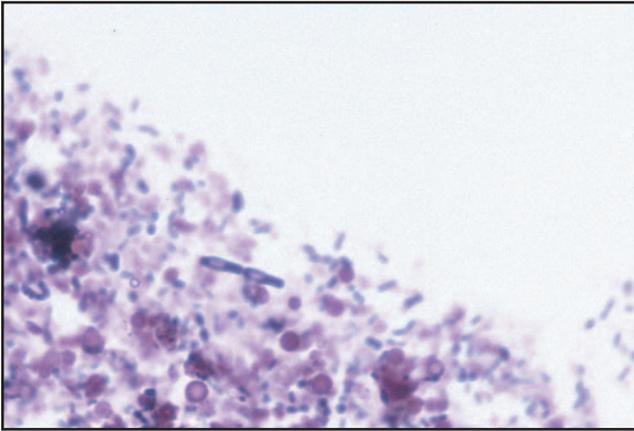


Fig 26.30 | A Gram's-stained smear of intestinal contents from a lory (*Trichoglossus haematodus*) that died of acute necrohemorrhagic enteritis. *Clostridium perfringens* was isolated from the intestinal tract. Note the large gram-positive rods with sub-terminal spores.

These should be opened to look for cecal worms and their contents should be included for culture. Cecal contents should be included in culture for *Salmonella*, especially in Galliformes.

Colon

Colon contents should start to look like fecal material as one moves toward the cloaca. Open the cloaca to look for papillomatous lesions, cloacoliths, trauma, inflammatory lesions and neoplasia (Fig 26.32).

In summary, intestinal samples should include the following: wet mounts from at least two different sites, smears for Gram's stain and possibly acid-fast stain, contents for aerobic and possibly anaerobic bacteria or *Campylobacter* culture, tissue for histopathology and ingesta for virology (direct electromicroscopy, virus isolation and/or DNA probes), and toxicology.

NEUROLOGIC

The brain and spinal cord can be very important in the diagnosis of some diseases, especially PDD. The dorsal calvarium should be carefully removed with rongeurs (Fig 26.33). Visualize the brain in situ for any obvious abnormalities such as abscesses, which should be cultured. Remove the brain by inverting the skull and transecting the ventral and cranial attachments (Fig 26.34). Collect a portion of the forebrain for virology and toxicology, and fix the rest of the brain in formalin. In neonates, the brain is so soft that making a cut through the dorsal skull and placing the entire calvarium containing the brain in situ into formalin is recommended. After fixation, the brain will harden somewhat and it can be removed more easily without damaging it.

A similar procedure can be followed for the cervical

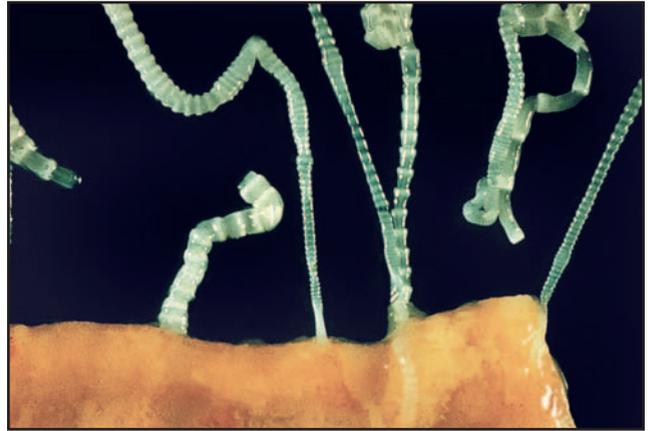


Fig 26.31 | Tapeworms attached to the intestinal mucosa of a great horned owl (*Bubo virginianus*).

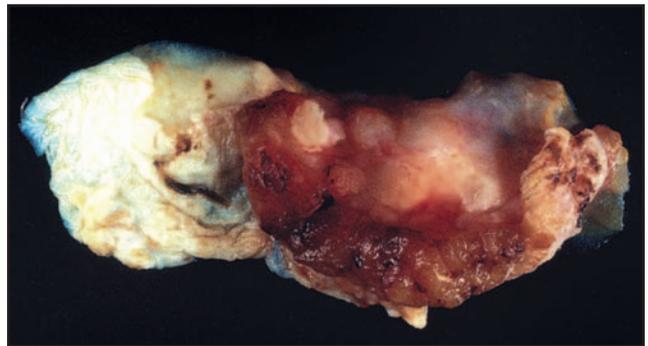


Fig 26.32 | Cloacal papillomatosis is seen in a lilac-crowned Amazon parrot. The cloaca has been opened caudally to cranially.

spinal cord. Cut the vertebral column with cord in situ into 2- to 3-cm pieces and fix in formalin overnight. This process will allow easier removal using rongeurs, with minimal damage to the less fragile, fixed spinal cord. In very small birds, cross-sections of the cervical vertebral column with the spinal cord in situ can be decalcified and examined histologically.

In birds with head tilt or neurologic disease, especially *Neophema* parrots and exotic finches, fix a large portion of the petrous temporal bone containing the middle ear. This bone can later be decalcified by the veterinary pathologist and sectioned to examine the middle ear for inflammation and viral inclusions associated with paramyxovirus infections. Congestion of the vascular sinuses in the bones of the skull is a common finding, but it is significant only if there also is corresponding subdural hemorrhage or bleeding of brain parenchyma.

The eyes can be removed and fixed in formalin if there is any suspicion of blindness, ocular or neurologic disease. Dissection through some orbital bone may be required for removal. Remember that the avian eye has bony scleral ossicles, which may make their sectioning somewhat more difficult. The globes can be transected at the optic nerves or dissection can be carried out

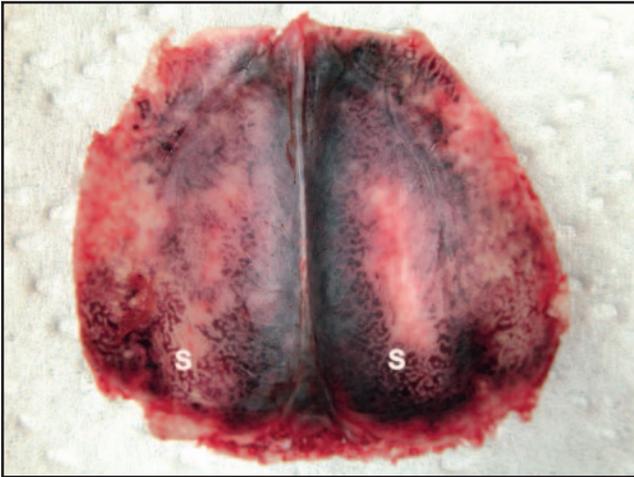


Fig 26.33 | View of the dorsal surface of calvarium of a psittacine bird with congested vascular sinuses (S).



Fig 26.34 | Dorsal surface of a normal brain from a blue-fronted Amazon parrot.

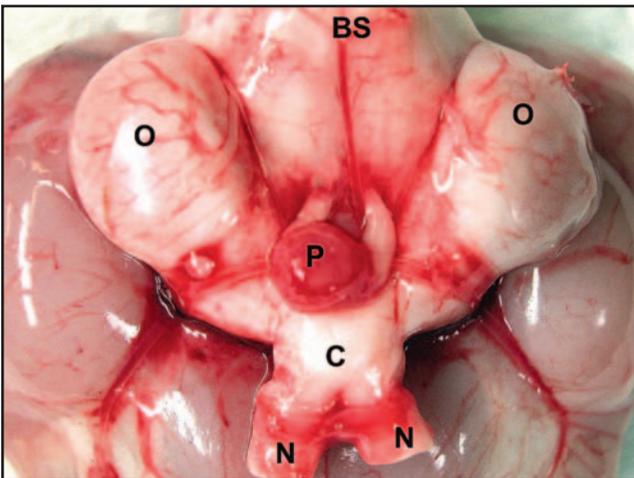


Fig 26.35 | View of the ventral surface of the brain from a blue-fronted Amazon parrot. Pituitary (P), optic lobes (O), brain-stem (BS), optic chiasm (C), optic nerves (N).



Fig 26.36 | A pituitary tumor in a budgerigar (*Melopsittacus undulatus*).

through the ventral calvarium to keep the optic nerves and chiasm intact and attached to the brain. On the ventral surface of the brain near the optic chiasm is the pituitary (Fig 26.35). Tumors of the pituitary have been reported in budgerigars and cockatiels (Fig 26.36).

MUSCULOSKELETAL

Bone marrow can be collected by aspiration of the femur and smears made and stained for cytologic evaluation. Collect a segment of femur using rongeurs and place it in formalin. Once fixed, the previously fragile bone marrow can be dissected out and examined histologically. Leukemic or aplastic processes can be diagnosed from bone marrow samples, and circovirus inclusions also may occasionally be seen histologically.

Samples of skeletal muscle should be collected for histopathology. Muscular lesions may include trauma, hemorrhage, degeneration, mineralization, and injection or vaccine site reactions. Myositis, degenerative myopa-

thy and *Sarcocystis* infection can be diagnosed histologically (Fig 26.37). Open the joints of the pelvic and thoracic limbs and look for exudate; collect synovial fluid with a sterile syringe for bacterial and mycoplasmal culture, although exudate also can be caseous.

Articular gout can be diagnosed by examining the exudate on cytology or by histopathology. The lesions of degenerative joint disease, periarticular proliferation and proliferative synovitis are fairly common in the joints of the feet, but also may occur in the shoulder, stifle and hock. Any bone or joint lesions demonstrated radiographically should be opened and sampled for culture and histopathology (Fig 26.38).

The flexibility of bones (eg, tibiotarsus, ribs) can be used in the assessment of the adequacy of mineralization. The bones should break with an audible snap if mineralization is normal. The rachitic “rosary” at the costochondral or costovertebral junctions and deformation of the keel or other long bones are obvious lesions of metabolic



Fig 26.37 | Pale areas in skeletal muscle from an emu (*Dromiceius novaehollandiae*) with degenerative myopathy. The heart muscle appears grossly normal.



Fig 26.38 | A proliferative osteosarcoma involving the right leg and pelvis of a peach-faced lovebird (*Agapornis roseicollis*).

bone disease. Sections of bone, especially areas of the metaphyses and epiphyses, can be examined histologically for metabolic bone disease.

Ratites are prone to developing angular limb deformities. The “rubber rhea” syndrome is often due to hypophosphatemic rickets. Other ratite limb deformities can be multifactorial, but nutritional imbalances in calcium, phosphorus and vitamin D₃, growth rates and problems with substrates are often implicated. Flock problems with limb deformities in ratites can be investigated through feed/forage analysis and bone ash analysis.

SMALL BIRDS AND DEAD-IN-SHELL

Necropsy of very small birds and neonates (under 15 g) is challenging. One can open the coelomic cavity and thorax and fix the entire body in formalin; opening the ventriculus as well is recommended for best fixation. The veterinary pathologist can then carry out dissection. It is very difficult to get good fixation of tissues in birds weighing more than 20 g, so this technique should not be used for them.

Dead-in-shell and egg necropsies can be performed, but there are limited lesions and testing available. Open the egg as aseptically as possible at the air cell end. Collect samples aseptically for bacterial culture, virology and DNA probes. Assess gestational age and positioning of the embryo or chick. Then place the embryos and membranes in formalin. Histopathologic diagnosis is often limited by autolysis, since the eggs often remain in the incubator for a period of time after embryonic death. Histologic examination of the blastodisk can help determine if the eggs are truly infertile or whether early embryonic death occurred post-fertilization.

Ancillary Testing of Samples Collected at Necropsy

After the necropsy has been concluded, the remaining parts of the carcass can be placed in a sealable plastic bag and frozen until diagnostic testing has been completed. Examine wet mounts of intestinal contents and crop or oral cavity scrapings as quickly as possible in-house for parasite ova, oocysts, motile flagellates, yeast and motile bacteria.

MICROBIOLOGY

Stain impression smears from organs and bone marrow, smears of exudate, or cells from fluid analysis with a Wright’s stain and examine for cell types and microorganisms, including bacteria and fungi. If bacteria are seen on the cytologic preparations, a stained smear can be destained and restained with Gram’s stain, or another smear can be stained. If macrophages with “ghost bacilli” are seen on the cytologic preparation, an acid-fast stain is in order to attempt to demonstrate mycobacterial organisms.

A Gram’s stain of the colonic contents can be performed in-house to provide a quick evaluation of the presence of abnormal bacterial populations, and then followed up with culture. This type of Gram’s stain is especially important in detecting possible clostridial organisms, which would then prompt an anaerobic culture. Avian gastric yeast also can be detected in fecal smears or from scrapings of the isthmus stained with cytologic stains.

Composite Samples

A collection of liver, spleen and air sac can be submitted

for *Chlamydoiphila* diagnostics. A Gimenez or Macchia-vello stain can be performed on impression smears of air sac, liver and spleen for the demonstration of elementary bodies. In Columbiformes, conjunctiva and nictitating membrane should be included, as elementary bodies may be confined to this location in these species. Fluorescent antibody and chlamydial culture may be available at certain laboratories, and some also can perform a DNA probe for *Chlamydoiphila* on a swab from the combined surfaces of liver, air sac and spleen.

Tissues, exudates or swabs can be submitted to diagnostic laboratories for bacterial, mycoplasmal or fungal culture as indicated. With the exception of samples for *Campylobacter*, which does not survive freezing well, these samples can often be frozen if not sent for culture immediately.

Special media is required for the culture of *Mycoplasma* spp. Alert the bacteriology laboratory if fastidious organisms such as *Campylobacter* spp. and *Bordetella avium* are of interest in the particular species or individual bird, as these organisms often require special media and incubation parameters. DNA probe testing for *Salmonella* spp. is available at some laboratories. An antibiotic sensitivity tailored to drugs used in pet avian species also can be requested if other birds on the premises are at risk.

Mycobacterial culture is required for accurate speciation of acid-fast organisms, and special media and handling are necessary. Once *Mycobacterium* isolates are grown on solid media, some laboratories are capable of speciating the organisms by the use of DNA probes and may offer antibiotic sensitivity testing for mycobacterial isolates.

Fungal culture may be requested in cases of suspected mycoses and is often required for accurate identification of the species involved. Antifungal sensitivity testing is available at specialized mycology laboratories.

A pool of parenchymal tissues (liver, spleen, air sac, lung, kidney, brain and bursa if present) and a separate pool of intestinal contents should be refrigerated or frozen for possible virus isolation or DNA probe testing. A combination swab from heart blood and the cut surfaces of liver, spleen, lung, kidney and bursa can be submitted for DNA probe testing for viruses such as psittacine circovirus and avian polyomavirus. Fluorescent antibody techniques on frozen sections of tissue may be available for certain viruses.

Polymerase chain reaction (PCR) tests are available for the detection of certain viruses, such as West Nile virus, on fresh or frozen tissues. It is important to contact the individual laboratory so the most appropriate tissues are

submitted, as this can depend on the particular virus or antigen and upon the particular laboratory's technique.

PARASITOLOGY

Direct wet mounts of intestinal contents and crop or oral cavity scrapings prepared and examined at the time of necropsy are invaluable in the diagnosis of trichomoniasis in pigeons, raptors and budgerigars; coxchlosomiasis in finches and canaries; and giardiasis in cockatiels and other psittacine and passerine species. Many of these organisms dry up easily, so examination should be performed promptly. Examination of these wet mounts under dark field or phase contrast, if available, may make the detection of flagellates and motile bacteria easier. Inoculating Diamond's media and submitting the media for incubation can attempt culture of some trichomonad parasites.

Microscopy of whole parasites such as nematodes, cestodes, flukes and acanthocephalans may provide morphology that can point to the classification of the parasites, plus characteristic ova may be visible within the helminths. An acid-fast or auramine stain can be performed on smears for the detection of *Cryptosporidium* oocysts, which can be found in the intestine, conjunctiva, nasal cavity or bursa.

A stained smear of the heart blood or lung impression can be examined for microfilaria and hematozoa such as *Plasmodium*, *Hemoproteus* and *Leucocytozoon*. Wright's-stained impression smears of lung, spleen and liver are especially important in canaries and finches for the diagnosis of the monocytic form of toxoplasmosis, as these organisms may not be visible histologically.

Rarely, flagellates can be demonstrated in impression smears from the lung, trachea, sinus and conjunctiva, which are not readily visible histologically. Other protozoal parasites such as *Sarcocystis*, *Toxoplasma* and *Leucocytozoon* can be found in impression smears of organs.

HISTOPATHOLOGY

Select a group of formalin-fixed tissues with lesions or a group of tissues that commonly contain histologic lesions that could lead to diagnosis and submit them for histopathology. This commonly includes tissues such as liver, spleen, air sac, kidney, lung, trachea, heart, bursa, brain, duodenum/pancreas and proventriculus/ventriculus. Save the remaining formalin-fixed tissues in case the diagnosis is not made with the first set of tissues.

This second set of tissues may include spinal cord, bone marrow, nasal cavity, skin and feathers, bone and joint, middle and inner ear, eyes, tongue, skeletal muscle, thyroid, parathyroid, adrenal, esophagus, crop, jejunum,

ileum, colon, ceca, gall bladder, ovary, oviduct, testes, thymus, nerve (ischiatric, brachial plexus) and beak.

The veterinary pathologist may recommend special diagnostics such as stains for acid-fast organisms, fungi, bacteria, iron or copper, depending on what is seen on the routine hematoxylin- and eosin-stained sections. In special situations, tissues may be embedded in plastic so that electron microscopy can be performed. Direct electromicroscopy also can be performed on intestinal contents or tissue homogenates. In situ DNA hybridization techniques on paraffin-embedded tissues are available for certain viruses such as Pacheco's herpesvirus, adenovirus, avian polyomavirus, psittacine circovirus and paramyxovirus.

Immunohistochemical stains can detect certain antigens from bacteria, fungi, viruses and parasites in paraffin-embedded tissues, and these techniques also can be utilized to detect some cell markers in the diagnosis of tumors. Gene sequencing of certain microorganisms (*Clostridium perfringens*, for example) in formalin-fixed, paraffin-embedded tissues is available at some diagnostic laboratories.

TOXICOLOGY

Toxicologic testing requires some idea of what toxin is being considered. This information often comes from the history and histopathologic findings. Contacting the toxicology laboratory is essential for submission of the most appropriate tissues and amounts.

The most common toxins tested for are heavy metals such as lead and zinc. Usually liver and kidney are required for this analysis, although zinc also accumulates in the pancreas preferentially. Heavy metals also can be detected in foreign bodies, water and feed. Copper accumulation in the livers of swans can be demonstrated qualitatively with special histologic stains for copper, but quantitative levels require toxicologic analysis.

Iron storage disease is most commonly seen in toucans, toucanettes, mynahs and birds of paradise; the condition is rare in psittacines, although there is emerging evidence that lorries and lorikeets may be prone to iron accumulation. The special histologic stain, Perl's Prussian blue, can provide qualitative information about the amount of iron in the liver, but, again, quantitative levels are detected by toxicologic analysis.

Poisonous plants can be found in the digestive tract and submitted to a botanist or university botany department for identification. The plants or wood can be frozen until submission to prevent the breakdown of toxic principles. Ingestion of fertilized plants can result in nitrate toxicity, and samples of these plants can be analyzed for the amount of nitrates present.

Polytetrafluoroethylene (PTFE or non-stick coatings) and other toxic inhalation products are rarely detectable in tissues, and the diagnosis is usually made by a history of exposure, the presence of pulmonary edema and hemorrhage, and the exclusion of other causes of death. There is a wide variety of items commonly found in the household that can give off PTFE fumes, including non-stick cookware and appliances such as self-cleaning ovens and electric grills.

Birds also can be sensitive to other inhalants such as carbon dioxide, carbon monoxide and fumes from glues, resins, plastics and paints. Mycotoxins may be implicated in the case of multiple birds suffering liver damage. Aflatoxins can be detected in foodstuffs, but usually by the time chronic liver damage is evident, the offending foodstuff is often no longer available. In the case of acute toxicosis, samples of the feed should be frozen along with liver and kidney, pending further investigation. Contact the toxicology laboratory for specimen requirements and costs.