



VII. POST-MORTEM PROCEDURES

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1. Introduction

Pathology is not a science for pathologists alone. The findings of the pathologist should be one of the bases of the clinician's understanding of disease and the pathophysiology of disease. Changes in anatomic pathology are the foundation of disease, and understanding these changes will give the clinician an advantage in selecting the right diagnostic tools and therapeutic approach.

Determination of the cause of death in zoo animals is often difficult and may require the close co-operation of a number of disciplines. Some zoo veterinarians perform a *post-mortem* examination in the zoo. But in doing the *post-mortem*, careless observation and sampling can result in useless, and sometimes even harmful, information. Those who perform post-mortem examinations in this manner are running a risk, as overlooking fundamental changes in tissue can be costly to both pocketbook and intellectual development (Cheville, N.F., 1999, *In: Introduction to Veterinary Pathology*, Iowa State University Press/Ames).

When a *post-mortem* is performed in the zoo itself, or samples are collected for diagnostic purposes, the results and information deriving from this activity are highly protocol sensitive. For a diagnostic laboratory to contribute fully to the final diagnosis, the specimen(s) collected must be selected carefully and preserved in suitable conditions. A thorough *post-mortem* examination of animals that die or are euthanised is a necessary adjunct to any good clinical practice.

The purpose of this chapter is to assist the zoo veterinarian in the performance of a thorough *post-mortem* examination and in the correct selection, preservation and transportation of pathological and biological specimens. This chapter will not be a complete protocol, but more of a guide. As with any activity, it is essential to prepare yourself before you begin. This includes reading and preparing your protocols and setting up an area or room, with the proper equipment, where you can perform the *post-mortem*. Keep in mind that the facilities need to be "infectious disease proof"; every *post-mortem* should be treated as an infectious problem until proven otherwise.

As the basis for this article we have used the excellent booklet, "Post-mortem procedures for wildlife veterinarians and field biologists" written by M.H. Woodford, D.F. Keet and R.G. Bengis (2000) and published jointly by the Office International des Epizooties (O.I.E.), Care for the Wild International and the Veterinary Specialist Group/Species Survival Commission of the World Conservation Union (IUCN). This little 55 page booklet is very comprehensive and should be present in every zoo where post-mortems are performed. It is available through the OIE, 12 Rue de Prony, 75017, Paris France (ISBN 92-9044-419-6).

We will not try to rewrite this booklet here, but several parts will be completely reproduced in this chapter, as is allowed by the OIE.

There are several reasons for performing a *post-mortem* or having one done. These can include: finding the cause of death, confirming a diagnosis, investigating unsuccessful therapy, increasing knowledge, or simply satisfying curiosity. In the zoo. Dead animals can also function as sentinels, indicating the presence of subclinical infectious diseases which may not be immediately related to the cause of death. Diagnostic pathology is not limited to a *post-mortem*. The pathologist uses the clinical history (including haematology, blood chemistry and therapeutic measurements), the gross description, culture results and other data, as well as the cytological and histological appearance of the lesions, to make a diagnosis. Absence of any of these, or incorrect submission of tissues, will hamper this process. And remember: it is better to store and preserve too much material than to realise after some time that the proper material required for a diagnosis has already been discarded.

We also consider it one of the functions of a “scientifically-minded” zoo to make optimal use of their animals and associated data. This includes gathering scientific information about the live animals regarding housing, feeding, behaviour, breeding, etc. Ideally, this would include maintaining a data bank of sera and organs or tissues, for future retrospective studies of diseases and their agents.

2. Submission of carcasses or specimens

When a zoo veterinarian has access to the services of a pathological institute that will perform the *post-mortem*, procedures should be established for the submission of the intact carcass. These specialists have the necessary experience and training, and their work will yield the best results. In some cases the zoo veterinarian or technician will perform the *post-mortem* examination and submit the appropriate tissue to a diagnostic laboratory. Based on the gross *post-mortem* findings, material will be collected and sampled for follow-up investigation. The quality of information received from such an examination is directly proportionate to the quality and choice of the specimens submitted and the information that accompanies them. When in doubt about “what” and “how” samples should be collected and packed, the laboratory should always be contacted before sending in any materials.

In many situations it is not possible to start a *post-mortem* immediately after death. To promote the rapid cooling of a small carcass, the fur or plumage should be thoroughly soaked with cold water to which a small amount of soap or detergent has been added to aid complete wetting of the coat or plumage and skin. The carcass should be placed in a plastic bag, all excess air squeezed out, the bag sealed or tied, and then refrigerated. Larger animals should be stored in a cool environment as soon as possible. Big animals will not cool down quickly enough to prevent extensive autolytic *post-mortem* changes and should be necropsied as soon as possible. When this is not possible, then opening the abdomen can be helpful in lowering the core body temperature.

The animal should be kept refrigerated until the *post-mortem* is performed or the carcass has been shipped to the laboratory. In general, providing the carcass has been cooled immediately upon death and can be delivered to the laboratory within 72 to 96 hours of the time of death, it should be refrigerated (**not frozen**). Small animals can be packed with sufficient ice or cool packs to keep the carcass cold until arrival at the laboratory. If delivery to a laboratory is expected to be delayed beyond 96 hours *post-mortem* the carcass should be frozen immediately rather than simply refrigerated. Frozen tissue specimens or carcasses must be packed with sufficient **ice** to keep them frozen until arrival at the laboratory.

Refrigerated or frozen specimens should be packed in a sturdy, insulated (Styrofoam®) box, preferably in a leak-proof sealbag, and shipped to the laboratory by a private courier service, which guarantees same- or next-day delivery to the laboratory.

Most laboratories cannot receive specimens over the weekend; it is thus advisable not to ship refrigerated or frozen specimens on Fridays or weekends. Remember, it is crucial that sufficient refrigerant be packed with the specimen and that it be adequately insulated to insure that it will remain cold (or frozen) until it is received by the laboratory personnel.

In instances where the carcass is extremely small, such as embryos, nestlings or very small adult animals, the entire carcass may be submitted for histological examination. This is best accomplished by opening the body cavity, gently separating the viscera and fixing the entire carcass in formalin solution.

When you perform a *post-mortem* yourself or collect diagnostic material it must be done systematically. The correct selection of material for further examination, and the correct sampling, storage and shipping of material, will increase the quality of results tremendously. A written report of the *post-mortem* findings will help the zoo veterinarian to keep track of the disease status of the zoo collection.

EVEN A NEGATIVE FINDING IS A FINDING, SINCE IT MEANS THAT THE LESIONS/CHANGES YOU WERE LOOKING FOR ARE NOT PRESENT.

3. *Post-mortem* site, protective clothing and equipment

For details see Woodford et al. (2000), Section I: Preparing for a *post-mortem* examination.

It is helpful to have a set of instruments designated for *post-mortem* examinations. These should be thoroughly cleaned and sterilised after use. A separate room to perform the *post-mortem* is also advisable. Instruments that are used for *post mortem* examinations should not be used for living animals. It is important to wear adequate protective clothing.

The **instrument pack** should include *post-mortem* knives, forceps, two scalpel handles (one for cutting, one for burning organ surfaces before taking a microbiology sample), stout scissors and/or poultry shears (for cutting bones), and fine scissors for dissection. Tiny animals such as finches, lizards or rodents require fine instruments such as iris scissors. For large animal *post-mortem* examinations special instruments such as a vibrating (cast-cutting) saw may be used.

Other useful equipment includes a (gram) scale, a hand-lens or dissecting microscope, and paper tissues.

In addition to instruments, one should have at hand:

- 10% neutral **buffered** formalin (= 4% formaldehyde),
- 70% alcohol for wetting and disinfecting the skin,
- 96-100% ethyl alcohol (for fixing specimens suspected of having gout, and 100mg/1ml for PCR testing),
- a bottle with normal saline (0.9% NaCl) with a pipette (for parasitological examination), and
- appropriate containers.

Other material for ancillary diagnostic procedures include:

- syringes and needles to obtain samples for serology, haematology, or cytology,
- clean glass slides for impression smears,

- a stain set for cytology (e.g. "Diff-Quick^R", Hemacolor[®], Stamp or Machiavelli),
- clean glass slides and coverslips for wet mounts (parasitology),
- burner for heating and sterilising a scalpel blade before taking a sample for microbiology,
- sterile swabs or culture tubes with appropriate transport media for bacterial, or fungal culture,
- transport media (96% ethanol or buffered 4 M guanidine isothiocyanate) for PCR testing (viruses, mycobacteria and chlamydia)
- petri dishes or freezer-proof tubes for submission of tissues for viral isolation.

It may also be helpful to have a camera available for documentation of gross lesions. A standard checklist and *post-mortem* report form will assist in recording observations.

4. Euthanasia

The method of euthanasia may affect specimens submitted to the pathologist. High doses of barbiturates are caustic to tissues and cause crystallisation in and on organs. Such changes may be mistaken for early gout, but will also change and mask macroscopic and microscopic lesions. When euthanasia solutions are used at an appropriate dosage, e.g. pentobarbital 200 mg/kg bw intraperitoneally or T61 0.5 ml/kg intramuscular, few alterations are seen. The euthanasia agent can also be administered intravenously, or into the spinal cord at the base of the skull with the head flexed (especially in larger birds). Administering such agents slowly to effect is helpful to prevent undesired artificial changes.

Serum or heparinised haematological samples should be collected prior to euthanasia. The blood may be centrifuged and serum or plasma submitted or saved and frozen pending *post-mortem* results. This may be helpful in diagnosis of endocrine disorders or viral infections. Routine haematological tests may also be performed on these samples.

5. Impression Smears

Impression smears of fresh cut organs or altered surfaces are not common practice at *post-mortems*. They are a useful and often underestimated adjunct to a complete *post-mortem* examination. In our protocol, two sets of impression smears are made at every *post-mortem* from liver, spleen, lung and rectum. Organs with pathological changes are automatically added to this list. For a first impression of the presence of bacteria, yeasts or protozoa, this technique is very useful. Tissue phases of parasites such as *Atoxoplasma* spp, *Toxoplasma* spp., *Plasmodium* spp, *Hemoproteus* spp., *Leucocytozoon* spp, and *Trypanosoma* spp. are mostly readily identified in impression smears of liver, spleen and lungs. Immunofluorescent staining for *Chlamydia* spp. can be carried out in specific laboratories on the impression smears of these organs in all *post mortem* examinations of suspected cases in reptiles and birds especially within the families *Columbiformes* and *Psittaciformes*. Immunohistochemistry staining on fixed paraffin-embedded histological sections is a good alternative when available. The now-days confirmation of Chlamydiosis is by PCR. Also, the cell-type of lymphoreticular and haematopoietic neoplasms is easier to diagnose from impressions of liver, spleen and bone marrow than from histology.

To make a good impression smear (actually a touch preparation), it may be easier to hold the slide when touching with the tissue. Grasp a small piece of the tissue with forceps so that a fresh cut, well-blotted surface faces downward. Lower the tissue to the clean slide touching it lightly. Retract quickly without dragging the tissue across the slide. Make several "touch preps" on each slide. Impressions are generally more useful when air-dried. If other fixation is necessary (e.g. heat fixation for acid-fast stains), it can be done after air-drying .

Exudate or any other fluids may be prepared for cytological evaluation by having a thick drop air-dried.

6. Fixation for Histopathology

Several philosophies may determine the choice of tissues for histopathological examination:

1. *Economic reasons*; these are poor grounds for decision-making, but in this case it is better to collect the tissues and, after consulting the pathologist, the selected tissues should be sent in but additional tissue samples should be retained "just in case."
2. *Completeness*; this is especially valid for a scientific, research situation. Collect all tissues listed in table 1.
3. *A standard selection* completed with a choice based on the *post-mortem* findings. This list is practical and will in most cases lead to sufficient diagnostic support. In table 1 the standard selection is marked with an asterisk (*).

Normally, selected tissues are fixed in **neutral-buffered formalin** for histopathological examination. If the formalin solution is not freshly prepared on a frequent basis, formic acid will be formed. A layer of pieces of marble at the bottom of the container or bottle will bind the formic acid to a precipitate, keeping the formalin neutral. This prevents the formation of "formalin pigment" in histological specimens; a confusing, annoying and unnecessary artefact that can be present in improperly fixed tissue samples.

Ten percent buffered formalin penetrates only about 2-5 mm in 24 hours, so specimens must be less than 10 mm thick. Penetration is slower in very bloody, dense tissues (e.g. congested spleen or liver) and more rapid in relatively porous tissue (e.g. lung). Formalin will not penetrate well into the brain through the unopened calvarium or into bone marrow unless the bone has been cracked. The biggest problem seen with submission of fixed tissues is inadequate fixation due to prior severe autolysis or an inadequate volume of fixative allowing continuing decomposition. Proper initial fixation is achieved if at least ten times the volume of formalin as volume of tissue is used. When preparing specimens for mailing the amount of formalin may be reduced after tissues have been fixed for 12-24 hours. Wet formalin-fixed tissue may be conveniently stored and shipped in heat-sealed plastic bags.

Other fixatives, such as those required for electron microscopy (EM), are not usually necessary, since formalin fixed tissue is easily refixed with glutaraldehyde and the main structures (including viruses) are preserved. For EM fixation very fresh tissue in tiny parts (1-2 mm³) is essential.

The number of tissue specimens submitted to the histopathology laboratory may depend on the cost per sample. If you do not send the complete set of specimens, it is prudent to save the rest in formalin while awaiting a diagnosis. If only grossly visible lesions or limited tissue specimens are submitted, a diagnosis may not be possible. When specific lesions are observed at *post-mortem*, the tissue specimens collected should include a small margin of normal tissue adjacent to the lesion. Too often, the limited tissue specimens submitted suggest a diagnosis, which cannot be confirmed because other tissues have already been discarded.

Tissue specimens for histopathology should not be frozen. Freezing creates crystals and ruptures cells, making histopathology virtually useless.

Tissues for toxicological analysis should be frozen. They may be frozen at -20°C after being wrapped in aluminium foil. The optimum temperature for freezing tissues for virus isolation is -70°C. If this cannot be accomplished, the tissues for viral isolation should be sent (by rapid mail) in sterile containers on wet ice to the laboratory.

For detailed information on sampling etc. see Woodford *et al.* (2000), Section III: "The collection and field preservation of biological and pathological specimens" and the Appendices.

Table 1: Tissues routinely collected for histopathology.

skin	thyroid glands	rectum	ovary & oviduct
(feather follicles)	parathyroid glands	caeca	testes (male)
trachea	oesophagus	(cloaca)	pectoral muscle
lung*	(crop)	spleen*	bone marrow
(air sac)	(proventriculus*)	liver*	thymus
heart*	stomach/ventriculus*	gall bladder	brain
kidneys*	duodenum*	pancreas*	spinal cord
adrenal glands	small intestine	(cloacal bursa*)	(ischiatric nerve)

* Standard selection of tissues for routine histopathological examination.

(..) Tissue specimens from birds. Selection of additional tissue specimens will depend upon gross lesions observed at post-mortem.

7. Autopsy protocol

There are probably as many ways to dissect an animal as there are pathologists. One should choose a procedure with which one is familiar and feels comfortable, and then use it consistently. No matter what procedure is used, each post-mortem should be performed in as regular and thorough a manner as can be accomplished by the prosector and a "complete" set of tissues and specimens be collected for subsequent histopathological, parasitological, toxicological, serological, and biochemical examination. The veterinarian should review the appended detailed checklist of organs to be examined, observations to be made, ancillary tests to be performed and specimens to be collected, prior to disposal of the remains.

The following procedure and checklist is used for avian species at the Diagnostic Pathology Laboratory of the Dutch Research Institute of Avian and Exotic Animals (NOIVBD) in Veldhoven, The Netherlands (www.noivbd.nl).

For a protocol for mammals see Woodford et al. (2000), Section II: "Post-mortem procedures".

Avian Necropsy Protocol

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Before starting the necropsy procedure (see also reference list), the packing material is to be inspected for the presence of mites or lice!

I. History

Read the history - including identification, physical findings, medical history and pertinent laboratory data - and summarise the most relevant data on your work sheet. Make a note of leg band numbers, transponders or other identifying marks.

II. External examination of the carcass

First make a carcass identification based upon identification: species, age, and colour pattern as well as leg band, tattoo or microchip implant data.

Record information about general bodily condition, weight, muscle mass, joints, integument (incl. beak and nails), plumage, (for defects, ectoparasites, faeces), body orifices (eyes, ears, nostrils and vent), uropygial gland, traumata, and abnormalities. Palpate the skeleton.

The feeding status can be judged based upon the muscles on the keel and the filling of the crop and intestines.

When heavy metals are suspected (e.g. rifle bullets or ingested lead) survey radiographs may be taken.

Examples (of alterations found at external examination):

- Broken feathers due to **feather picking**; diagnosis: normal feathers on the head.
- Altered feathers with constrictions at the base caused by **PBFD**; diagnosis: histology of skin with feather-follicles; PCR test.
- thickened dry skin caused by **Malassezia** sp; diagnosis: cytology skin scraping.
- Look for feather and skin parasites.
- Swelling above the eye or dilated nostrils with a plug in parrots due to **vitamin A deficiency**; diagnose: histological examination with metaplastic changes in salivary glands.
- Conjunctivitis and sinusitis related to **ornithosis, chlamydiosis or psittacosis**
Conjunctivitis with **pox-lesions**; diagnosis: cytology, histology and culture.
- Abdominal or other swellings, **tumours, egg-related peritonitis**: diagnosis: histology
- Cloacal mucosal prolapse, **papilloma**; diagnosis: histology.

III. Preparation of the bird

Small birds are wetted and plucked, all other birds should be wetted with alcohol 70% before the necropsy. This is done to allow better visualisation of the skin, to part the feathers to permit incision of the skin and to prevent loose feathers from irritating or harming the prosector (zoonosis) or contaminating the viscera.

The bird is positioned on its back, in small birds the wings and legs are pinned to a dissecting board with nails or needles, large birds are fixed on a metal tray with pieces of rope.

IV. Post mortem examination

General remarks:

- use a gram-scale for body weight and measuring the size of organs,
- open all tube-like structures,
- cut all parenchymatous organs in slices to find small focal lesions,
- tissue for optimal formalin fixation should preferably not exceed 3 to 4 mm in thickness (5 mm maximum),
- ratio of tissue to formalin required for adequate fixation is 1:10,
- collect tissue samples during the necropsy to prevent desiccation. Do not wait till the gross examination is finished,
- remember to collect and submit specimens from a broad spectrum of organs and systems,
- collect at least heart, lung, liver, spleen, kidney, gonad, and adrenal, and a piece of intestine (duodenum and pancreas) for histopathology,
- when suspecting a viral problem, collect 100mg tissue in 1ml ethanol 96% for a PCR, freeze tissue as soon as possible at -20°C, or collect tissue on wet ice till shipment.
- when you suspect a bacteriological problem, make a impression smear and **look** before you select you culture media or send an organ or swab to a laboratory for culturing.

1. **An incision is made in the skin along the ventral midline from the mandible over the sternum to the cloaca.** The skin is reflected to expose the subcutis, crop, pectoral muscles, keel, abdominal wall, leg muscles and fat. Watch for colour of the muscles, parasites, haemorrhages, and oedema. Judge the amount of food in the crop. In pigeons a vascular plexus in the deep layers of the cutis of the cervical region can be seen, the *plexus venosus intracutaneous collaris*. This plexus can be mistaken for an extensive haemorrhage.

Examples

- *Stripes in leg- or breast-muscle; **sarcosporidiosis**; diagnosis: cytology of such a stripe reveals the bradyzoites.*
 - *A large dark spot distal to the keel; **swollen liver**: diagnosis: see under 3.*
 - *Changes of the skin; **cnemidocoptes, yeast-infection**; diagnosis: wet mount and cytology smear.*
2. **Make an incision through the pectoral muscle along the sides and around the posterior border of the sternum through the abdominal muscles; cut with heavy rongeurs, scissors or poultry shears through the ribs, coracoid bones, and clavicle to remove the sternum.**

Examine the inside of the sernum, the air sacs and pericardial sac, and make impression smears (when abnormalities or inflammations are seen). During dissection of the keel the air sacs are easily seen. Normal air sacs appear as glistening transparent membranes.

Examples

- Opaque air sacs or (fibrinous) inflammation: **chlamydiosis**; diagnosis: cytology with special staining, PCR
- Opaque air sacs or obvious inflammation: **bacterial infection**; diagnosis: rods or cocci in cytology smear; culture and sensitivity test.
- Air sacs covered with white/yellow plaques: **fungal infection**; diagnosis: wet mount (heated with chlorallactophenol), showing hyphae, culture.
- Air sacs solid with white/yellow material; **chronic fungal infection, mostly aspergillosis**; diagnosis: wet mount showing hypha, culture.
- Air sacs, esp. cervical and prescapular, with small black dots in passerines and small psittacines; **Sternostoma tracheocolum infestation**; diagnosis: magnifying-glass and wet mount.
- Air sacs filled with food: **forced feeding**; diagnosis: wet mount and histology.
- Pericardial sac filled with fluid: **inanition, cachexia**; diagnosis: muscle wasting, oedema and gelatinous fat-tissue.
- Pericardium covered with white chalky deposits: **visceral gout**; diagnosis: wet mount with crystals; often in combination with nephritis.

3. **Identify the (para)thyroids cranial and lateral to the syrinx along the carotid arteries.** Remove the thyroids when required. Look for the thymus along the neck in juvenile birds. The liver is examined in situ (examples see 5).

Examples

- In budgerigars **enlarged thyroid glands**; diagnosis: histology.
- Parrots (especially African greys) **hyperparathyroidism**; diagnosis: histology.
- "Abscesses"; **Salmonella or E.coli infections**; diagnosis: rod shaped bacteria in cytology, culture.

4. **Remove the heart with the carotids and thyroids attached and cut across the apex to check for an "open" lumen and to assess the thickness of the ventricle walls.** Open the heart and large vessels and examine the valves and endocardial surface. Keep in mind that the right atrioventricular valve in birds is a muscular structure.

Examples

- Yellow plaques on the wall inside the large vessels; the vessels are stiff: **atherosclerosis**; diagnosis: macroscopic (gross) examination, histology.
- Epi- or endocardial haemorrhages: **septicaemia or agonal event**; diagnosis: continue post mortem.
- Gelatinous, serous pericardial fat; **starvation, chronic illness**; diagnosis; continue post mortem.
- Changes (inflammation, necrosis) in the myocardium: **myocarditis**; diagnosis: cytology, histology, microbiological isolation, continue post mortem.
- Cardiomyopathy with muscle cysts: **sarcocystis**; diagnosis: cytology, histology.
- An enlarged lumen of the left ventricle and only slight difference in thickness of the ventricle walls: **heart failure**; diagnosis: congestion of the lungs and/or liver.

5. **Examine and measure the liver.**

Take a sample for cytology and histology. Decide if you want to do a bacteriological culturing or freeze piece of liver-tissue for virological or toxicological testing.

Separate the rest of the liver from the viscera by holding the ligaments in the forceps and cutting them with scissors, examine the gallbladder (if present). For a thorough examination, slice the liver at regular intervals.

Examples

- *Enlarged red variegated liver with pale areas: **hepatitis**; diagnosis: cytology with many inflammatory cells; histology.*
- *Enlarged liver with necrotic areas: **hepatitis by chlamydiosis, herpesvirusinfection**; diagnosis: imprints, culture, histology, PCR.*
- *Very extensive acute liver necrosis: suspected for **peracute or acute hepatitis by bacterial septicaemia, polyoma-, herpes- adeno- or reovirus**; in juvenile African grey parrots **acute circovirus infection** diagnosis: macroscopic (gross) examination, cytology, histology, virology (PCR), culture.*
- *Focal yellow proliferation with often central necrosis; **tuberculosis**; diagnosis: see above.*
- *Small round yellow necrotic foci: **salmonellosis or yersiniosis**; diagnosis: imprints with rod-shaped bacteria; culture.*
- *Evenly enlarged, often variegated, pale liver: **leucosis**; diagnosis: macroscopic (gross) examination; included often other organs; cytology and histology.*
- *Evenly enlarged, often variegated, pale soft liver: **degeneration**; diagnosis: cytology hepatocytes with vacuoles; histology.*
- *Enlarged orange, yellow liver: **fatty liver**; diagnosis: macroscopic (gross) examination, cytology, histology with Sudan III stain.*
- *Liver with necrotic ulcer: **histomoniasis (black head)**; diagnosis: histology.*

6. **The spleen can be found by cutting the oesophagus in the bifurcation** of the trachea and with combined blunt and sharp dissection remove the viscera leaving the lungs and kidneys.

Do not cut the cloaca, but bend the viscera caudally. This exposes the spleen in the angle between the proventriculus, gizzard (and liver). Examine, remove and measure the spleen; make impression smears from a fresh cut surface after blotting to remove excess blood.

Examples

- *Spleen-swelling together with air sac opacity: **chlamydiosis**; diagnosis: see above.*
- *Very large swollen and cherry red spleen in parrots watch for **herpesvirus infection (= Pacheco's) or sarcocystis**; diagnosis: liver necrosis with intranuclear inclusion bodies or protozoa, cytology, histology, IFT, PCR, virus isolation.*
- *Very large swollen and cherry red spleen in penguins and some other species; **Plasmodium infection**; diagnosis: cytology for parasites in macrophages and severe pneumonia, histology*
- *Swollen and pale: **(bacterial) septicaemia**; diagnosis: cytology with bacteria, culture.*
- *Multiple irregular yellow foci in the spleen; **tuberculosis**; diagnosis: the same foci in other organs, in imprint non-staining rods, acid-fast. Differentiation avian/bovine strains by culture or PCR.*
- *Large firm spleen: **tumour**; diagnosis: histology.*
- *Enlarged friable spleen with multiple, millitary necrotic foci: **salmonellosis, yersiniosis**; diagnosis: the same foci in liver and caeca; imprint with rod shaped bacteria; culture.*
- *Homogeneous red enlarged spleen in canaries and finches: **atoxoplasmosis**; diagnosis: cytology.*

- Small, grey spleen: **lymphoid depletion**; stress, viral infection; diagnosis: cytology, histology, virus isolation.

7. **Examine the adrenals, gonads (determine sex) and genital tract, and the kidney with ureters *in situ*.** Remove the kidneys by applying gentle traction to the cranial vessels. Notice the adrenals and look for the sciatic nerve in the middle division of the kidneys. In our laboratory the kidneys are not routinely screened in cytology, but only when pathological changes are seen.

Examples

- A swelling inside the oviduct: **egg-binding, egg concrements**; diagnosis: open the oviduct.
- Irregular swellings related to kidney or gonads: **tumour**; diagnosis: macroscopic (gross) examination and histology.
- Pale swollen kidneys with white striation: **urate congestion**; diagnosis: dehydration; histology (fixation 100% alcohol!!).
- Irregular pale swollen kidney with white foci: **nephritis with "renal gout"**; diagnosis: histology (fixation 100% alcohol).
- Irregular swollen kidney with multifocal abscessation: **bacterial infection**; diagnosis: histology, cytology, culture.
- Enlarged red kidneys: **acute nephritis**; diagnosis: histology.
- Pale swollen friable kidneys: **kidney degeneration**; diagnosis: histology.
- White, firm small kidneys: **chronic kidney fibrosis**; diagnosis: macroscopic (gross) examination
- NB the adrenals are important for the histological diagnosis of proventricular dilatation disease (PDD, avian bornavirus infection) in psittacines.

8. **Free the lungs by applying gentle traction to the trachea and oesophagus** and cut the attachment to the ventral ribs and backbone at the thoracic inlet. This may be difficult as there is no pleural space in birds. Using blunt and sharp dissection will free the lungs. Inspect the lungs. Open the oesophagus. To open the syrinx, trachea and main bronchi a strip has to be cut out; cut through the lungs at intervals; make an impression smear from the lungs.

Examples

- Dark coloured grey lungs: **lung oedema**; diagnosis: on cut surface clear serosal fluid.
- Dark coloured wet red lungs: **lung congestion**; diagnosis: from a cut surface only blood; the lungs are supple and evenly bright red; watch for congestion in other organs and alterations of the heart. Think also of polytetrafluoroethylene (Teflon^R) toxicosis, acute mycotic infection, Plasmodium and sarcocystis.
- Dark firm lungs often variegated and focal changes: **pneumonic foci**; diagnosis: cut surface; cytology (inflammation cells); histology.
- Dark, supple, dry lungs: **atelectasis**; diagnosis: on cut surface only a dark colour of the surface of the lung and dried up.
- Scattered through the lungs white/yellow foci: **aspergillosis, tuberculosis**; diagnosis: wet mount with hyphae (aspergillosis), acid fast rods (in routine quick staining, non-stained rods) (tuberculosis); culture and histology.
- Irregular scattered pneumonic foci: **bacterial pneumonia**; eg. Salmonella spp. or Yersinia spp.; diagnosis: cytology and culture.
- In the syrinx of parrots white material: **syringeal mycosis**; based on a metaplasia due to vitamin A deficiency; diagnosis: see aspergillosis.
- In the trachea red worms: **Syngamus spp**, black dots: **Sternostoma mites**; mucous and fibrin: **avipoxvirus, cytomegalovirus**

9. Examine the "abdominal" viscera.

Open and inspect the proventriculus and gizzard (with koilin layer). Examine the contents for foreign bodies and heavy metals.

The bowel should be opened by making cuts at intervals to examine the contents and the wall for changes and parasites. Open and inspect the caeca when present. Look for the pancreas, bursa and umbilical sac.

Take the following samples:

- > contents from the duodenum and rectum for parasitology (including direct examination on very fresh specimens for flagellates as well as other techniques)
- > contents from the rectum for a smear for staining (Diff Quick®)
- > contents from the rectum for microbiology

Examples

Crop

- Thickened wall with white material: **yeast infection**; diagnosis: smear of the material; culture.
- Thickened wall with mucous material: **capillaria infection**; diagnosis: smear of scraping of the epithelium; histology.
- Thickened wall with grey/yellow material, sometimes with trapped air bubbles: **trichomoniasis**; diagnosis: wet mount; cytology; histology.
- Local yellow necrotic ulceration: **pox-lesions**; diagnosis: macroscopic (gross) examination; histology; virusculture.
- Local red mucosal thickening: **papillomas**; diagnosis: histology.

Stomach (proventriculus and ventriculus)

- Dilated proventriculus and gizzard, often stuffed with seeds (sunflower): **gastric dilatation syndrome**; diagnosis: histology; (ganglio)neuritis, lymphoid infiltrates in the adrenals..
- An empty proventriculus with excess of mucous: **Macrorhabdes ornithogaster (formally "megabacteria")**; diagnosis: wet mount and cytology.
- Swollen red glands in proventriculus: **Tetrameres spp**; diagnosis: parasitologic examination.

Intestines

- Haemorrhagic contents duodenum: **coccidiosis**; diagnosis: wet mount, cytology.
- Haemorrhagic, black contents in the entire small intestine: **haemorrhagic diathesis**; diagnosis: history (fasting during high energy need for over 24 hours), macroscopic (gross) examination.
- Pseudomembraneous covering of the duodenal wall: **hexamitiasis**; in cranes; diagnosis: wet mounts, cytology and histology.
- Thickened wall with or without blood in the lumen: **enteritis**; diagnosis: wet mount and cytology; parasitology; microbiology. Beware: in psittacines very rarely coccidia, often **ascaridia**; in small passerines rarely worms, often **coccidia** spp.
- Haemorrhagic contents: **lead intoxication, clostridium infection, pseudomonas infection, Giardia spp.**; diagnosis: lead in gizzard; lead analysis liver and kidneys; cytology, culture.
- Clear watery contents in small intestine with flabby wall: **hexamitiasis**; diagnosis: fresh wet mount, cytology, histology.
- Yellow non-digested starch and broken seeds in small passerines: **Cochlosoma or Campylobacter spp.**; diagnosis: fresh wet mount, cytology, selective culture.
- Enlarged caeca with pseudomembraneous to necropurulent content; **typhlitis**; diagnosis: galliformes: **histomoniasis ("blackhead")**; diagnosis: cytology, histology (often with liver lesions)



- *Caeca with nodular lesions: **parasitic typhlitis**; pheasants: **Heterakis isolonga**; diagnosis: worms and ova; histology*

Cloaca

- *Congested, swollen red mucosa: **papilloma**: diagnosis: histology.*

Bursa

- *Especially in young birds for detecting virus infections e.g. **circovirus**: diagnosis: histology, PCR.*

BEWARE: TRY TO ESTABLISH A RELATIONSHIP BETWEEN THE CLINICAL HISTORY AND THE POST-MORTEM FINDINGS

10. **Open the anterior part of the oesophagus from the beak, make a wet mount.** Remove the tongue and cut the salivary glands. Inspect the beak, choanae and oesophagus.

Examples

- *Tongue with yellow "abscesses" at the location of the salivary glands in psittacines: **metaplasia, due to vitamin A deficiency**; diagnosis: wet mount, diet history, histology.*
- *see also crop/intestines (eg. trichomoniasis, avipox, candidiasis).*
- *Chronic, necrotic lesions especially in commisures: **tuberculosis**: diagnosis: cytology (acid fast stain), histology, culture.*

11. **Cut across the beak through the nostrils and sinuses.**

Examples

- *The presence of turbid mucus: **sinusitis**; diagnosis: wet mount, cytology, culture.*

12. **Inspect the joints, bones, bone marrow, brains.**

Joints of the wings, legs and feet should be opened and examined. If exudate is present, cytology should be done as well as a microbiological examination. White, chalky deposits may represent urate deposition.

Bone marrow is most easily collected from the tibiotarsus for both cytology and histology. In bone marrow tubercular lesions can be found; often visible on X-rays.

Ecchymoses within the calvarium are a common agonal change and do not imply head trauma.

Examination of the nervous system and associated tissues is governed by the presence or absence of neurological or ocular disease.

The brain may be removed by deskinning the head, making a sagittal incision through the calvarium and removing the bony calvarium to expose the brain. When sampling for histology, it is often better to leave the brain inside the skull, after opening it, and immerse the whole head in formalin.

13. **The muscles of the legs and the sciatic nerve** running on the posterior surface of the femur should be examined.

V. Final activities

1. **Bacterial cultures** are done from the liver and the rectum and all abnormal organs, especially when bacteria are seen in the imprints!
The following media are selected: blood agar, selective Enterobacteriaceae agar (brilliant-green-agar) and serum broth.
The intestinal contents are collected in tetrathionate broth as an enrichment medium for *Salmonella* spp. When special microorganisms are expected (e.g. anaerobes, *Campylobacter* spp.) contact the laboratory.
2. When a **mycotic problem** is suspected a Malt-agar, or other selective culture medium, is selected as well.
3. The **impression smears** are allowed to dry, stained with 'Hemacolor^{R1}' or "Diff Quick^R" and Stamp or Macchiavello (for Chlamydia), and examined by microscope with objective 100x in immersion oil.
The slide for an IFT for Chlamydia is fixed in cold acetone (freezer -20°C) and sent to the laboratory.
4. POSITIVE cytology for Chlamydia requires sampling for IFT or PCR.
5. Examine the **wet mounts** of gut contents.
6. Samples collected for **ancillary diagnostics** should be packed, labelled and stored properly, until shipment. See that each sample is provided with the essential documentation .
7. Make a detailed report and use this to document the samples.

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Necropsy report form and check list

The following checklist can be use during the post-mortem examination as well as writing the necropsy report:

1. Bird species, weight, age/leg band number, sex, summarized history.
2. Date of necropsy, your name.
3. Macroscopic (gross) examination:

External examination

- general bodily condition: muscle mass: robust, well muscled, moderately muscled, thin, emaciated, depot fat
- feathers/integument/ectoparasites
- palpate skeleton
- body openings/oral cavity

Internal examination

- fat/subcutis/body wall
- body cavities (air sacs/pleura/peritoneum)
- (para)thyroids, thymus
- spleen (size)
- heart, aorta, other vessels
- liver, gall bladder, bile ducts
- reproductive system (gonads, repr. tract)
- urinary tract (kidneys, ureters) and adrenal glands.
- respiratory tract (nasal/sinus, choanal, larynx, trachea, syrinx, air sacs, lungs)
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- digestive tract (beak, tongue, oropharynx, oesophagus, crop, proventriculus, gizzard, duodenum and pancreas, small intestine, yolk sac, caeca, rectum (colorectum), cloaca, bursa of Fabricius, vent)
- special senses (eyes, ears, nares)
- musculoskeletal system: muscles, skeleton (sternum, ribs, vertebrae, long bones), bone marrow, joints
- brain, pituitary, spinal cord, meninges, peripheral nerves

Wet mounts (crop, rectum, etc.)

Cytology (liver, spleen, lung, rectum)

Chlamydiosis

TENTATIVE (DIFFERENTIAL) DIAGNOSIS

Ancillary diagnostics: bacteriology, mycology, virology, parasitology, toxicology, others

Tissue saved:

Tissues submitted for histopathology: