

Chapter 19

Suggested Check List of Equipment for Clinical and Necropsy Examinations

Laboratory Specimen Submission Manual 8th Edition
NSW Agriculture Veterinary Laboratory Services

1. Personal

Rubber boots
Overalls
Gloves
- disposable
- postmortem gloves or gauntlets
Postmortem apron (optional)
Towel and soap
Wet weather gear

2. Animal Husbandry

Halter
Nose grips
Bleeding choke rope
Twitch
Pig handler
Drugs
- Rompun 2%
- Xylocaine 2% etc

3. Clinical

Stethoscope
Thermometers
Obstetrical gloves

4. Clinico-pathological

Test strips eg urine, blood and glucose
Nitrate and cyanide test

5. Clinical Specimens

Vacutainers – plain, heparin and EDTA
Vacutainer needle holders and needles (18G)
Sterile containers (100ml)
Sterile bottles – yellow top plastic 5, 25, 50ml
Swabs – packets sterile
Transport media

- Amies charcoal transport medium in bottles or commercial swab packs
- Phosphate buffered glycerol saline (PBGs) in bottles – supplied by labs

Microscope slides (and spreaders for blood films)
Syringes (1-20ml) and needles (14-26G)
Scalpel handle and disposable blades
Scissors
Non-sterile faecal containers (50-100ml)
Vaginal mucus pipettes
Ethanol/iodine for skin asepsis
Biopsy instruments and small bottles of fixative

6. Euthanasia

Rifle and ammunition
Euthanasia solution and syringe/needle
Killing knife

7. Necropsy

Knives

- 20cm skinning 2.5cm wide straight blade
- 20cm skinning 2.5cm wide curved blade
- 15cm boning knife pointed straight

Steel

- 30cm Butcher's

Footrot secateurs

- 7.5cm blades – Wilkinson Sword

Handsaw 30cm blade

- Sandvik (Sweden) and replacement blade

Scissors

- Mayo straight 16cm
- Mayo straight 14cm
- Double sharp 11cm fine
- Pointed/1 rounded end and knob (for gut running)

Bone forceps

Toothed forceps

- 20cm
- 18cm
- 13cm

Scalpel handles

- No. 4

Scalpel blades

- No. 24 and/or NO. 20

Rib Cutters

Meatsaw/hacksaw

Cleaver/hatchet

Buckets – plastic (2)

Trays

- plastic (large)
- plastic (small)

Sterile scissors and forceps for virological tissue samples

8. Pathological specimens

Sterile containers

- 50ml
- 25ml
- 5ml

Plastic bags 0.1mm thick

- large
- medium
- small

Rubber bands

Microscope slides and diamond pencils, or markable slides and pencil

Histopath jars with 10% neutral buffered formalin

- large 500ml
- medium 250ml
- small 125ml

Special fixatives (eg Bouins) if required

Ball of string

9. Decontamination/Disinfection

Container and 20 litres water

Disinfectant – litre Lysol or similar

Nail scrubbing brush (for cleaning instruments)

Long handled scrubbing brush (for cleaning boots)

Roll paper towel

Plastic garbage bags

Used beer can for contaminated "sharps" or disposable sharps container

10. Clerical

Clip board

Postmortem and laboratory submission forms

Specimen collection handbook

Pencil

Marking pens

Black biro

Adhesive labels

String-tie labels

11. Storage

Large metal esky with 1 blood vacutainer box and small foam esky inside

Frozen cold bricks, dry ice or portable car fridge

Serum storage plastic disposable tubes – 5ml

PRACTICAL SESSIONS - POSTMORTEM TECHNIQUES FOR RUMINANTS

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To quote Dr HG Belschner "there is perhaps no way by which a veterinarian or stock owner can obtain more information about disease in animals than by a properly conducted postmortem examination".

Before starting an autopsy a thorough clinical history of the flock or herd is essential to get some idea of the diseases involved. Begin the autopsy with the intention of examining first the system which clinical signs suggest is the seat of the disease but always complete the full autopsy examining all organs in a logical and systematic order.

The ideal postmortem specimen is a freshly dead animal, preferably one that has been sacrificed while showing terminal clinical disease. Where possible the carcass should be placed on a plastic sheet or a clean grassy area to minimise dust and other contamination.

Before starting, it is essential to have all your equipment organised and readily accessible. Suggested requirements include:

- knives and sharpening steel
- secateurs
- scissors
- forceps
- butchers saw (for removing brains)
- large secateurs (for cutting ribs)
- water with disinfectant
- formalin container
- sterile jars
- string
- plastic bags
- trays
- a micro-cassette tape recorder or note paper for recording your findings
- scrubbing brush
- soap and towel

Protective clothing including overalls, rubber boot and gloves should be worn when doing the autopsy. It is a good idea to have water with antiseptic on hand to clean the instruments during the autopsy.

For ruminants lie the animal on its right side. If the animal was found dead, note the position in which the animal was found, any signs of struggling, any discharge from external orifices and any evidence of jaundice, external wounds or injuries.

Open the carcass systematically by first removing the forelimb by severing the pectoral muscles and brachial plexus and laying the leg back followed by opening the hip joint of the hind limb by severing the teres ligament to lay the leg back. Incise the midline and reflect the skin followed by skinning the neck and head to give complete removal of the skin from the left side of the carcass. Next open the abdominal cavity along the lower flank and note any fluid which may be present. The abdominal organs can be observed *in situ* and need not be disturbed at this stage. The diaphragm is then observed and incised to confirm the negative pressure normally present in the pleural cavity. The rib cage is then removed using the large secateurs or rib cutters. The viscera of the left side of the carcass is now fully exposed. Note any evidence of oedema, subcutaneous congestion, haemorrhages, icterus, dehydration, emaciation or any other general body changes. With the carcass now opened the organ or systems indicated by the clinical disease may be examined followed by all other systems in the body.

The gastrointestinal tract involving the stomachs and intestines is removed as one by cutting the dorsal mesentery and then tying the oesophagus and cutting it as it passes through the diaphragm. These can all be placed on a tray for further examination later. Check the spleen on top of the rumen, particularly if in a recognised tick fever or Anthrax area.

The remaining abdominal organs are examined systematically and samples taken for laboratory examination where appropriate.

The liver should be examined for changes in colour, size, shape and any external abnormalities then incised to check texture and consistency. The size of the gall bladder and the nature of its bile contents should also be noted. If indicated a slice of liver no thicker than 2cm should be submitted to the laboratory for histopathology while a portion should also be submitted for bacterial culture if a bacterial disease is suspected.

The urogenital system should be examined by checking size, shape and colour of the kidneys, noting the thickness of the capsule and any external abnormalities of the renal cortex. Kidneys should be incised in a sagittal plane checking for deposits or other abnormalities in the renal pelvis. Specimens for laboratory examination should be taken where appropriate. The second kidney, the ureters, bladder and urethra should also be examined.

The thoracic organs are removed intact by cutting the vessels at the base of the heart and then tying the proximal trachea and following it down through the thoracic inlet and removing the heart and lungs as one. The lungs are examined for shape, colour, consistency, inflation and elasticity, then the trachea opened down into the bronchi to check for evidence of pneumonia or other abnormalities. The heart and pericardium are examined intact then opened to check for abnormalities in the external myocardium. The heart is opened in the direction of blood flow from posterior vena cava to aorta with heart valves being closely examined.

The oral cavity including the teeth and tongue should be examined then the mandibular symphysis split and the tongue, pharynx and larynx removed through the mandibular space for closer examination. The endocrine glands particularly the thyroids on the lateral surface of the trachea and adrenals anterior to the kidneys should also be examined. The musculo-skeletal system should be examined by incising muscles and opening joints where indicated.

Examination of the brain is essential for the diagnosis of central nervous system diseases. In the course of the autopsy, the skin will have been removed from the head, face and upper neck. Sever the head from the neck at the atlanto-occipital joint after first obtaining cerebrospinal fluid from this site, should it be required.

A meat saw is used to remove the cranial cap. First cut transversely across the skull at the level of the orbit. Next two lateral cuts from about the level of the inside orbit caudally and outwards then across to the occipital condyle on each side. The cranial cap is levered upwards from the back of the occipital condyles and the meninges cut to expose the brain free of its enclosing membranes. Tilt the skull backwards and allow the brain to drop out gently after cutting the optic chiasma and other cranial nerves. For histopathology the brain is immersed in 10% buffered formalin ensuring that the volume of fixative is at least ten times the volume of tissue. Check the base of the cranium for abnormalities.

Returning to the gastrointestinal tract the omental fat is cut off the rumen and the abomasum. The stomachs and intestine can then be separated and tied with string in two places then examined individually. The rumen should be opened along its greater curvature and the internal lining examined for mucosal sloughing erosions, abscesses or such. The rumen contents should be checked particularly when a poisoning is suspected. The reticulum and omasum are then incised and their internal linings examined. The abomasum should be incised and the mucous membrane checked for oedema, inflammation or erosions suggestive of parasitism or other irritations. Where parasitism is specifically suspected the abomasum and intestines should be submitted unopened direct to the laboratory or contents saved for worm counts in the field.

The intestines should be examined and the nature of the contents noted. The intestines may be opened at various places to check for inflammation of the mucosa or take smears from the wall. For histopathology small sections, preferably 2cm cylinders, should be taken with the ends nicked to cause outward curling of the mucosa when it contracts in the formalin. Alternatively, intestines can be opened and spread out on a thin cardboard to prevent twisting and curling.

Special note should be made of the mesenteric lymph nodes to check for enlargement or oedema suggestive of an inflammatory reaction.

On completion of the autopsy, specimens for the laboratory should be labelled, equipment disinfected and washed and the operator scrubbed down. Finally make sure you record the postmortem findings immediately whilst still at the site. Micro-cassette tape recorders are useful for making notes and a more detailed postmortem report can be written later.

PRACTICAL SESSIONS - POSTMORTEM TECHNIQUES WITH SPECIAL REFERENCE TO SHEEP

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Introduction

There is nothing "mystical" about conducting a postmortem examination, however for it to be instructive and useful requires a methodical, orderly and consistent approach. With experience, a definitive autopsy can usually be completed in 10-15 minutes. It is an exercise in observation and deduction, which normally allows definitive conclusions to be drawn. Most tissues can only respond in a limited and consistent way to a host of deleterious stimuli. Recognition of the altered state often permits a reasonable estimate as to its cause. Confirmation may come from more sophisticated laboratory back-up, but a provisional diagnosis is at least possible from gross postmortem findings.

Postmortem Equipment

General equipment

Comparatively little specialist equipment is required. Suitable protective clothing, including disposal gloves should be worn, both for personal protection and to reduce the chance of disseminating infection to other properties. Soap and water, and disinfectant are required for personal clean-up afterwards.

A good quality butcher's knife is a pre-requisite. While size and shape may be a matter of personal preference, it is imperative that the knife be sharp. A steel and a small oil-stone are needed to keep it so. Become adept at their use. Disposable scalpels are useful for detailed dissection, as are a pair of medium-sized scissors and forceps. String is useful for tying off portions of intestines, etc. Horticultural pruning shears can be used as rib cutters. Footrot shears are suitable as bone forceps, as in removing spinal cords in lambs and young sheep, but do not supply sufficient mechanical advantage for the removal of spinal cords from the vertebral columns of adult sheep. A small hand-saw can be used to remove the brain from the cranium. Where a brain or spinal cord is required, the head or vertebral column can usually be taken to a site (such as a veterinary laboratory) where powered-tools are available to facilitate removal and examination.

Ancillary Equipment

Useful information can be obtained from urinalysis by multitest "dipstick" urine tests. Similarly, "dipstick" pH indicators are available to measure abomasal and rumenal pH. Material to be transported over some distance or during hot weather should be kept chilled, and requires

proper packaging. Assorted sized plastic bags are useful and their contents need to be well labelled. Where specimens could leak in transit, double-packaging is advisable. Special transport media for microbiology is often required; use of sterile swabs and containers for routine microbiological sampling reduces the risk of chance contamination of samples.

Tissues harvested for histopathology, should be promptly preserved. Ten per cent formol-saline is the routine fixative (preservative) of choice. Abomasum and intestinal tract can also be fixed in Bouin's fixative. While Bouin's solution acts quickly, it penetrates tissues poorly and so is unsuitable for solid tissues. Hollow-tubed viscera such as intestines should be partly incised lengthwise to allow adequate penetration of fixative to the inner surface. Tissue samples from solid organs such as liver, kidney and spleen should not exceed 1cm thickness or 3cm along any other edge, for proper fixation. Long, thin sections (<3mm) often curl up on fixing, and are difficult to process.

Remember the golden rule:

Ten Volumes Of Fixative Are Required To Preserve One Volume Of Tissue

Preliminary Examination

The aim of any field investigation is to provide clients with useful information on which they can act. Before commencing any autopsies it is always highly instructive to place the disease episode under review in its proper perspective.

You need to be aware of the specific circumstances surrounding the cause for the present investigation. Many times, "having asked the right questions", a provisional diagnosis can readily be established even before a detailed postmortem examination is begun. The axiom, "common things occur commonly", should always remain uppermost in one's mind. It is important to know which common diseases are present in any locality, and their seasonality.

Preliminary information required relates to number, age and type of sheep involved, duration of the problem, duration of the clinical course, whether there are any particular feeding or management practices peculiar to the affected sheep, and what recent treatment, if any, has been given. Information on routine Clostridial disease vaccination and anthelmintic control programs is important.

Dead and sick sheep should be examined critically before detailed postmortem examination to assess their physical condition, whether they died quietly or after struggling, whether there are any discharge present, and the rate/extent of postmortem decomposition. Clinical blindness, weakness, ataxia, jaundice, diarrhoea and respiratory distress can provide valuable clues as to the ultimate cause of losses. Elevated rectal temperature may indicate acute infection. If possible collect clotted and

unclotted blood samples (use EDTA anticoagulant for preference) from affected live sheep and unaffected flockmates. Follow-up clinical pathology at a diagnostic laboratory provides potentially valuable information on the circulatory system, general nutrition status, trace element-mineral or vitamin-abnormalities and, from enzyme or metabolite assays, the functional status of liver, kidney, musculo-skeletal system, etc.

Preliminary studies show that in many ways anterior chamber fluid from the eyes reflect serum values of some enzymes, metabolites and electrolytes. Postmortem blood coagulation and haemolysis rapidly invalidate many serum assays, however anterior chamber fluid values reflect antemortem blood values for some time (hours) after death, and can provide useful diagnostic information (unpublished data).

Guidelines for Dissecting Sheep

Conventionally, sheep are autopsied in left lateral recumbency (ie left side down). The rumen then lies underneath rather than over other abdominal viscera. In left lateral recumbency the spleen is however virtually inaccessible. For systemic virus infections, where uncontaminated splenic tissue may be of paramount importance for virology, it is best to ignore convention, and autopsy the sheep in right lateral recumbency!

The right handed operator faces the abdomen of the sheep when it is in left lateral recumbency. Reflect the upper front and hind leg, disarticulating the hind limb at the coxo-femoral joint. Reflect the skin on the right side of the body, and on both the right and left sides of the neck and face. Cut the right side abdominal muscles, following the line of the ribs, the lumbar muscles and continuing to the anterior midline attachment at the pubis of the pelvis. The muscle mass is hinged in the midline.

It can either be reflected or removed entirely. There is less likelihood of puncturing abdominal viscera if the abdominal muscles are removed in this way, rather than by commencing with a ventral midline incision.

The tongue, oesophagus and trachea are dissected and reflected to the thoracic inlet. Expose and examine the thyroids, attached to the proximal trachea. The right side of the diaphragm is cut away from the ribs. (That side of the lungs will deflate unless the trachea has been plugged or clamped). The right half of the rib cage is removed by cutting through the ribs at the costo-chondral junction near the sternum, and close to their insertion in the vertebral column. Horticultural pruning shears make good rib cutters for sheep. Check for pliability and fragility of the ribs. Bone quality here is reasonably representative of bone quality in the remainder of the skeleton. The symphysis of the mandibles is cut and the jaws retracted laterally to expose the molars and hard and soft palate. The head can now be disarticulated and severed at the

atlanto-occipital joint before the brain is removed. Hock and knee joints at least should be inspected as well as any other grossly abnormal joints. Reflect the skin before opening the joint. Open the pericardial sac and check for excessive pericardial fluid.

At this stage, major dissection has been completed and most internal organs are accessible for detailed inspection. Before commencing this systematic examination, it is important to overview the visible organs in situ, noting any abnormality of size, shape, colour and position. Provided adequate care has been taken, any extraneous or excessive body fluids present can be related to an abnormality in the animal, rather than as a contribution from the operator.

It is a matter of judgement as to when specific tissues are harvested for further processing, however tissues and fluids for microbiology should be collected as soon as practicable to minimise extraneous contamination.

The carcase is now systematically eviscerated. As they become available, hollow viscera should be opened to allow inspection of the inner surfaces. This applies particularly to trachea, bronchi, heart and major vessels, alimentary tract, bladder and reproductive organs.

The oesophagus is dissected free and the heart, lungs and trachea are removed and inspected. The abomasum is transected where it emerges from the reticulo-rumen and at the pyloric junction with the small intestine. The large intestine is transected at the rectum. The viscera should be transected between 2 ties or clamps to prevent spillage of gut contents. The abomasum is removed by careful traction and blunt dissection; the small and large intestines are removed in their entirety, by careful dissection from the liver and by cutting the roots of their mesenteries. The pancreas is embedded in the small intestinal mesentery.

The intestinal tract should be stripped from its mesentery. Abomasum and intestines can now be examined in detail and processed for a total worm count if desired (see separate section). The oesophagus and forestomachs can now be removed. The spleen normally remains attached to the lateral wall of the rumen.

Remaining internal organs can now be examined, including urinary bladder, kidneys, reproductive organs, adrenals and liver. Muscle masses of limbs and back should be incised and inspected.

While the above is not a detailed anatomical dissection guide it does provide a simple, systematic approach to autopsy of the sheep, with the technique generally applicable to non-avian domestic animals. (Conventionally, non-ruminants are autopsied in right lateral recumbency, rather than left lateral recumbency). Judging the normality of various organ systems is a matter of experience. Detailed descriptions of gross alterations associated with various disease states are recorded elsewhere in this publication.

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