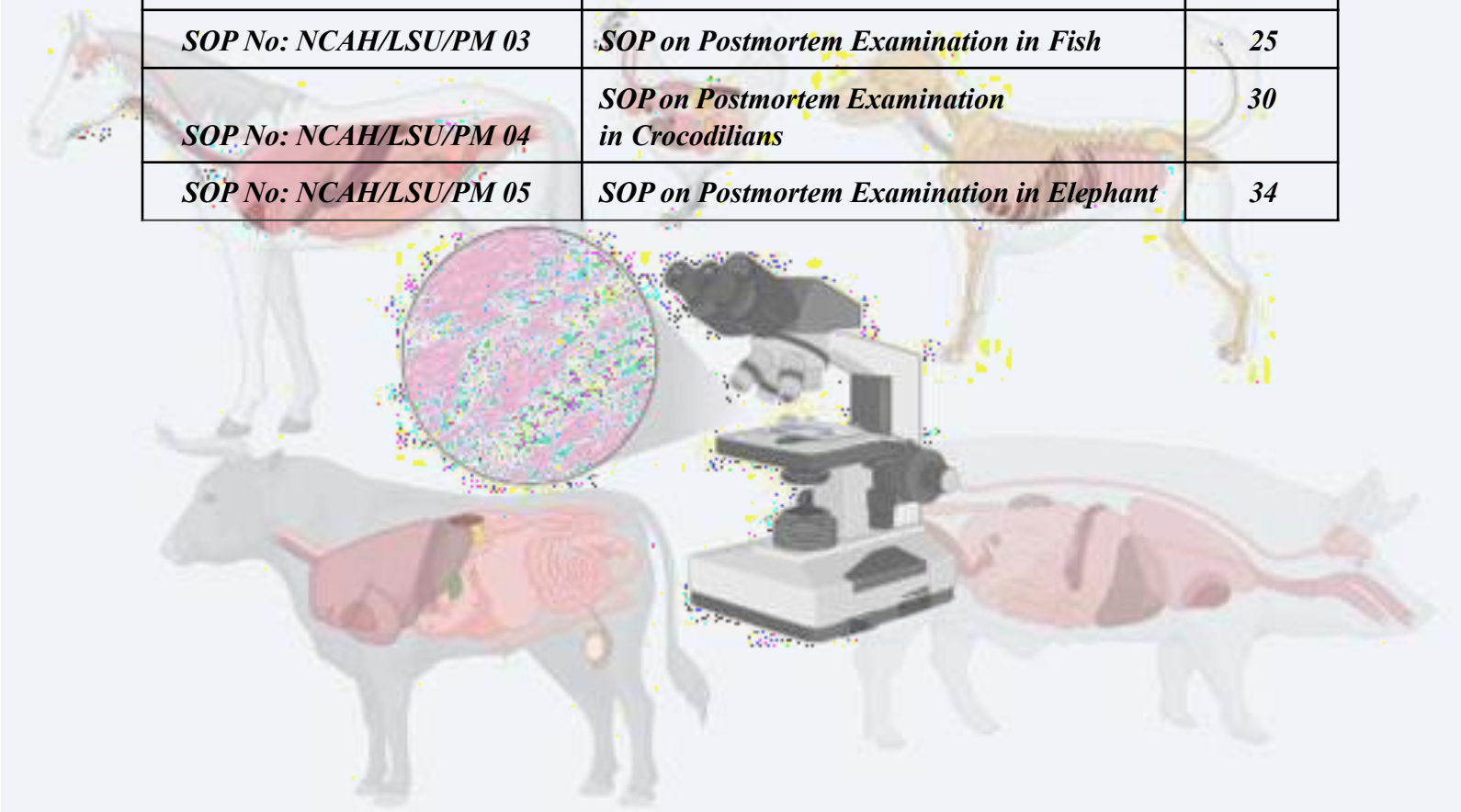

Checklist for Acceptability of Carcass for PM

Section	***Criteria***	***Yes (✓)***	***No (✗)***	***Remarks***
General Condition	*Carcass is fresh and free from decomposition*	☐	☐	
	Normal color; no abnormal discoloration or emphysema	☐	☐	
	Was the carcass frozen, preserved in formalin or bleach?	☐	☐	
Identification & Traceability	*Carcass properly identified (tag/mark)*	☐	☐	
	History and clinical findings	☐	☐	
	Details (date/time/batch) of Death	☐	☐	
Physical Integrity	*Carcass intact without excessive mutilation*	☐	☐	
	Essential organs (liver, lungs, heart, spleen) available for inspection	☐	☐	
	No damage interfering with examination	☐	☐	
Suitability for Examination	*Suitable for visual inspection*	☐	☐	
	Suitable for palpation and incision	☐	☐	

SOP No: NCAH/LSU/PM 01
Title: SOP on Postmortem Examination in Mammals
Version No: 2, Total Pages:16
Issue Month/Effective Date: May 2026
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Supersedes Version No: 2
Prepared by: Postmortem Section, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi
Approved by:
Application/Distribution: NCAH, NVH, RLDC, RVH&EC, SVL, DVL, DoFPS

1. Scope

Postmortem in animals is a vital diagnostic tool in veterinary science. It involves the systematic examination of a deceased animal to determine the cause of death, identify disease processes, and assess the effectiveness of treatments. The scope includes detecting infectious, nutritional, toxic, and management-related conditions, which helps in disease surveillance, herd health management, and prevention of future outbreaks. Postmortems also contribute to research, teaching, and legal investigations in cases of suspected malpractice or poisoning. By providing insights into pathology, they guide veterinarians and farmers in improving animal welfare, productivity, and public health through better disease control strategies.

2. Objective

To determine the cause of death and gather useful information for veterinary science. It helps identify diseases, trauma, or poisoning, and detects infectious agents that may threaten other animals or humans. Postmortems also evaluate treatment effectiveness, provide forensic evidence in cases of cruelty or malpractice, and contribute to education, research, and disease prevention.

3. Purpose

3.1. To outline the proper procedures for performing a necropsy and post mortem examination in livestock / wild animals.

3.2. Necropsy is to be performed for diagnostic purposes, disease outbreak, research and also vetro-legal cases.

4. Responsibility

4.1. Necropsy must be performed by Veterinarians, Veterinary para-professionals/Lab Technicians on cadavers or carcasses that died due to disease or on research animals.

4.2. Necropsies must be performed by investigators and staff when unexpected morbidities or mortalities are observed in a herd or flock especially in the farms.

5. Apparatus

5.1. PPE: Rubber boots, protective clothing, rubber apron or lab coat, rubber gloves.
5.2. PM set: Knives – large and small, with sharpening steel.
5.3. Scissors (various sizes) and saws; bone cutters
5.4. A number of sterile, open-mouthed screw top jars of different sizes for specimen collection
5.5. Sterile swabs in air-tight/leak proved screw capped test tubes
5.6. Alcohol cleaned slides for smears, preferably in a rack or box
5.7. Spatula
5.8. Plastic bags for specimens
5.9. Petri dishes
5.10. Labels
5.11. Soap, water, disinfectant and towel.

6. Reagents, solution and buffer

1. 10% Formalin or Buffer Neutral Formalin

2. 70% alcohol for wetting and disinfecting the skin,
3. 96-100% ethyl alcohol (for fixing specimens suspected of having gout, and 100mg/1ml for PCR testing),
4. Normal saline (0.85% NaCl) with a pipette (for parasitological examination),
5. Disinfectant

7. Procedure

7.1.Preparation

7.1.1. Animal necropsy should be performed in areas specifically designated for that purpose in the laboratories or identify an ideal location in the farms or the areas.

7.1.2. Proper personal protective equipment and attire must be worn when performing necropsy. Disposable gloves, shoe covers, and gown must be worn when conducting a necropsy.

7.1.3. Full protective clothing must be worn when handling animals infected with biohazardous materials or chemical carcinogens and includes double gloves, cap, disposable gown, shoe covers, mask (or respirator if required), and eye protection.

7.1.4. Obtain and record the animal and/or herd history.

7.1.5. Make sure appropriate disinfectant is available.

7.1.6. Carefully note external abnormalities, Check orifices, genitals and mammary glands. Make note of any injuries, wounds, parasites. Observe the general appearance of the carcass: rigor mortis, nasal and anal tissues, wounds, enlargements, eyes, skin lesion, condition of flesh and visible mucous membranes.

7.2. Necropsy procedure

7.2.1. Ruminants, Swine, Equines

7.2.1.1. Cattle, sheep, goats, and pigs are best positioned on their left side. Horses should be positioned on their right side.

7.2.1.2. Make an external examination and place ectoparasites in 70% Ethanol. Conduct a thorough external examination by reviewing the body surface and orifices for abnormalities. Palpate for superficial swellings, or for enlarged organs or masses within body cavities.

7.2.1.3. Collect nasal swabs and skin lesions or swabs, if required. Place in transport or growth nutrient media, and refrigerate.

7.2.1.4. To prevent contamination, disinfect the skin or use clean instruments to open body cavities. Open the abdominal and thoracic cavities carefully so as to prevent contamination from the outside or from a cut organ.

7.2.1.5. Observe, but do not disturb, organ placement, noting any abnormalities. Examine organs and tissues *in situ* before dissecting or collecting tissues and record any abnormalities.

7.2.1.6. With a syringe, aseptically collect a specimen of any abnormal body fluid

7.2.1.7. Aseptically collect specimens of liver, kidney, spleen, and lymph node (gastrohepatic node for swine).

7.2.1.8. Aseptically collect specimens of lung and heart.

7.2.1.9. Remove the tongue, open the pharynx, and collect the tonsil (swine).

7.2.1.10. Remove the trachea, lung, and heart. Collect tracheal and bronchial swabs, if appropriate. Examine the respiratory tract and heart.

7.2.1.11. Tie off and remove a 3" section of ileum just anterior to the ileocecal valve. Double ligate to prevent spillage of intestinal contents. Do not tie off intestinal segments to be placed in formalin because the fixative should infiltrate the lumen of the organ.

7.2.1.12. Complete the examination of the abdominal cavity. The entire digestive tract should be opened.

7.2.1.13. Decapitate the animal, remove the brain and collect specimens, noting any abnormalities.

7.2.1.14. Indicate on the necropsy form which tissues are collected or sampled that are to be submitted for other tests.

7.2.1.15. All lesions that were observed during the examination, or that are observed during the necropsy must be recorded on the appropriate necropsy form, and include a complete description (e.g., size, number, color, shape, texture, severity, and weight or volume as appropriate) as far as possible.

7.2.2. Dogs & Cats

General procedures of necropsy in Dogs and Cats are the same as above. In addition, the procedure for removal of brain has been detailed below:

7.2.2.1. Make an incision through the temporal muscles on each side, on a line from the centre of the foramen magnum, through a point 1/2 inch above ear canal and onto the frontal bone.

7.2.2.2. Saw just through the bone along these lines, being careful not to damage the brain.

7.2.2.3. Now see transversely through the frontal bone just above the orbit, meeting the two lateral incisions, lift off the top and remove the brain.

7.2.2.4. Examine the meninges and the brain itself.

Refer Annexure for pictorial illustrations

8. Risk assessment

8.1. If it is suspected of anthrax or rabies as the cause of death, or in the cases of unexplained sudden death, the carcass must never be opened. If anthrax is suspected, a blood smear slide taken from a cut on the ear is sent to the labs for confirmation. If rabies is suspected – the head/brain sample should be removed intact and sent to the lab in a sealed container. For small animals the whole carcass can be sent.

8.2. In both cases, protective clothing, including boots, gloves, face mask and goggles must be worn. The carcasses should be burned or deep buried under stones with quick-lime.
8.3. All carcasses should be handled with care especially if they were known to present risk of zoonotic disease.
8.4. No eating, drinking, grooming, or other activities that are a means of exposure are permitted in necropsy areas.
8.5. To limit the risk of unexpected or unknown exposure, all workers handling carcass must be offered vaccination for rabies (in endemic areas).
8.6. Transport unfixed tissues in leak-proof containers.

9. Waste disposal

9.1. Decontaminate instruments before cleaning them.
9.2. Clean and disinfect all work surfaces or the premises where necropsy was conducted.
9.3. Decontaminate self (e.g., disinfect and remove boots, gloves, and coveralls).

10. References

- University of Minnesota, Veterinary Diagnostic Laboratory, Standard Operating Procedure (2016).
- University of Queensland, School of Veterinary Science, Standard Operating Procedures (2017).
- University of South Florida, Standard Operating Procedure, Necropsy and Post Mortem Examination (2012). SOP#: 018.2
- USDA, Animal and Plant Health Inspection Service, National Veterinary Services Laboratories, Guidelines for Necropsy

11. Annexure:

11.1. Postmortem Steps for Ruminants

Step 1:

Expose the thorax and abdomen

Lay the animal in left lateral recumbency. Reflect the right forelimb and hindlimb. Carefully incise abdominal muscles to expose the abdominal organs without rupturing the intestine or forestomachs. Using rib cutters, cut the rib cage along the ventral and dorsal aspects to expose the thoracic contents

Step 2:

Expose the oral cavity

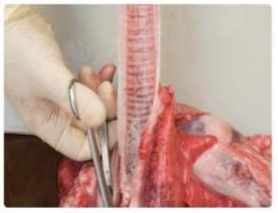
Extend the incision up the neck to the chin. Using rib cutters, cut through the mandibular symphysis to expose the oral cavity. Check for lesions

Step 3:

Dissect the neck

Hold the tongue and dissect through the hyoid apparatus to release the pharynx and larynx. Continue dissecting the oesophagus and trachea down to the thoracic inlet

Step 4:



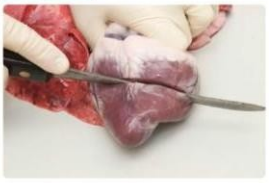
Open the trachea

Using scissors, cut down the trachea extending along the major brochi into the lungs. Check for the presence of lung worms

Step 5:

Sample the lungs

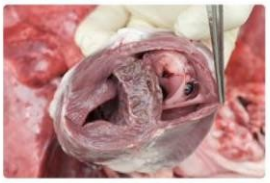
Step 6a:



Dissect the heart

Make two transverse sections (1 cm apart) two-thirds of the distance from the apex of the heart

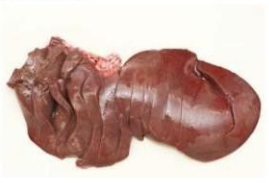
Step 6b:



Dissect the heart

Examine the A-V valves through the exposed ventricles. Sample the heart

Step 7:



Dissect and sample the liver

Make multiple slices through the liver to detect any lesions not grossly visible. Sample the liver

Step 8a:

Dissect the intestines

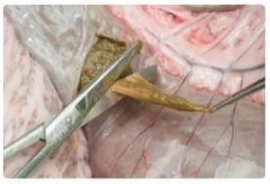
Sample the duodenum and jejunum

Step 8b:

Dissect the intestines

Expose the ileo-caecal junction by lifting the small intestines over the dorsal aspect of the carcase. Sample the ileum and ileal contents

Step 8c:



Dissect the intestines

Sample the caecum and colon

Step 9a:

Examine the abomasum

Examine the abomasum for the presence of Haemonchus parasites, hyperplasia or nodular changes. Sample the abomasal tissue

Step 9b:

Examine and sample the rumen

Test rumen pH (Normal 5.5-7.0). Examine the contents for intact leaves of poisonous plants. If ARGT is suspected, sample rumen fluid

Step 9c:

Examine and sample the forestomach

Empty the forestomachs and examine the ventral rumen mucosa for evidence of rumenitis. The ventral pillars are often affected. Sample the rumen

Step 10:

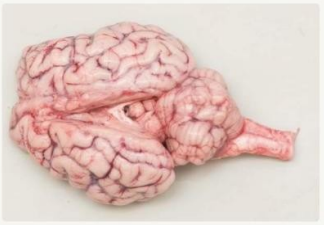
Examine and sample the kidneys

Step 11:

Examine and sample the muscles

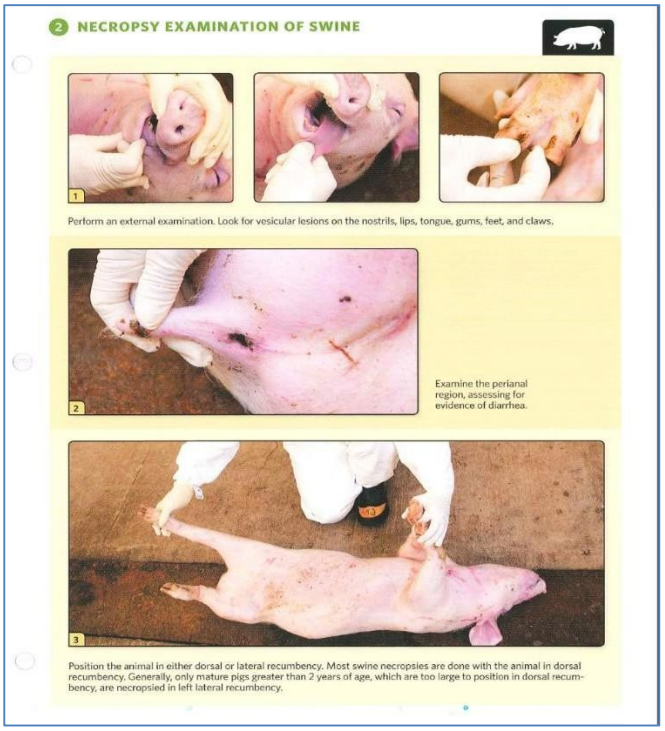
Slice hindlimb musculature looking for areas of pallor suggestive of nutritional myopathy. Sample the skeletal musculature

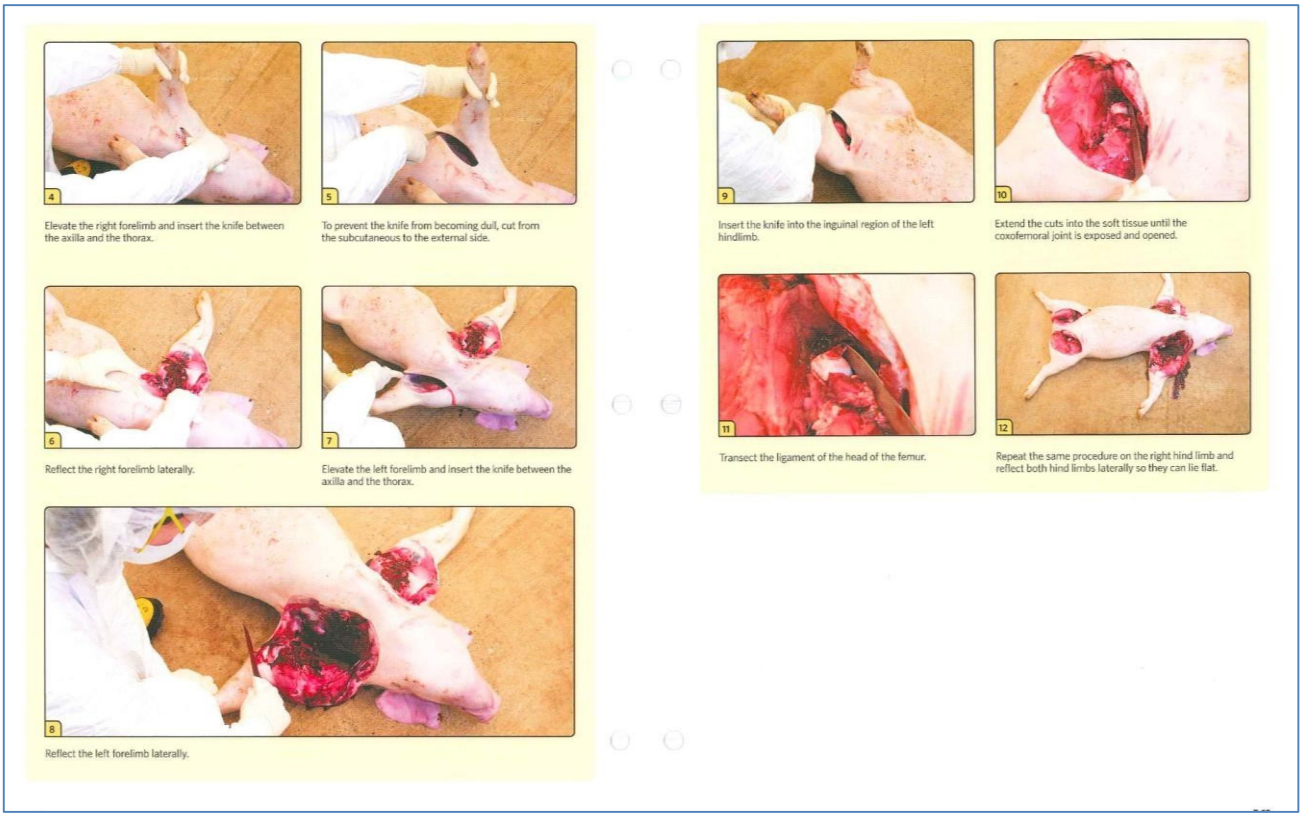
Step 12:

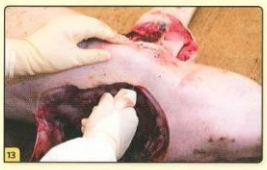


In some circumstances, the brain and spinal cord are required for diagnostic purposes. Refer to the brain removal techniques section of this guide.

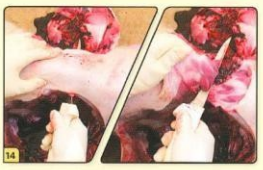
11.2. Postmortem steps for Swine



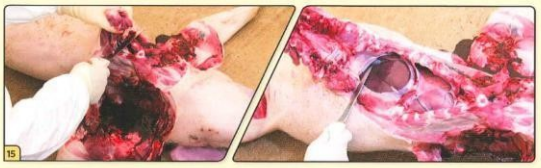

4
Elevate the right forelimb and insert the knife between the axilla and the thorax.
5
To prevent the knife from becoming dull, cut from the subcutaneous to the external side.
6
Reflect the right forelimb laterally.
7
Elevate the left forelimb and insert the knife between the axilla and the thorax.
8
Reflect the left forelimb laterally.
9
Insert the knife into the inguinal region of the left hindlimb.
10
Extend the cuts into the soft tissue until the coxofemoral joint is exposed and opened.
11
Transect the ligament of the head of the femur.
12
Repeat the same procedure on the right hind limb and reflect both hind limbs laterally so they can lie flat.



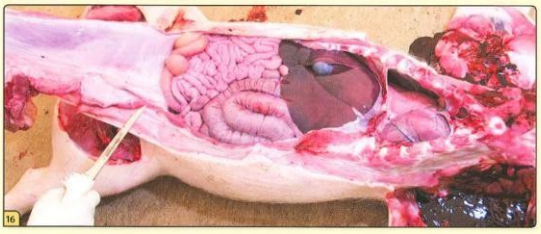
In younger pigs, the thoracic cavity can be entered by removing the sternum. Begin by inserting the knife (sharp blade facing cranially) beneath the skin over the manubrium.



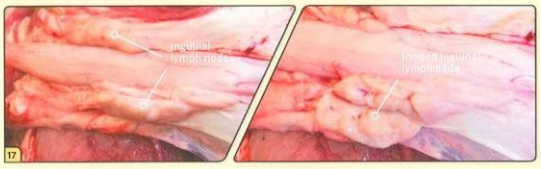
Rotate the blade ventrally and cut the skin.



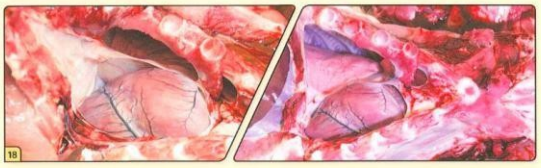
Starting at the manubrium, cut along the costochondral junctions of the ribs, working your way to the caudal thorax.



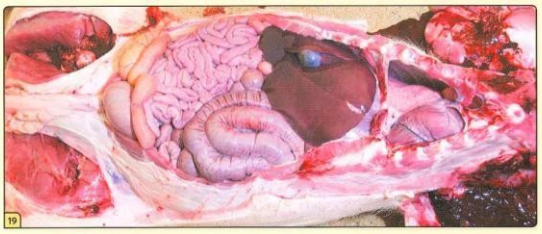
Continue the cuts caudally to the level of the inguinal incisions.



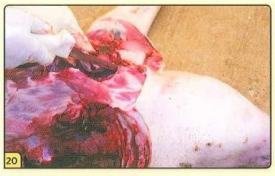
Identify the inguinal lymph nodes, located caudally on either side of the reflected abdominal flap. Incise and exam the lymph nodes.



Cut the mediastinum and pericardium to expose the lungs and heart for visual inspection. Examine the thoracic viscera *in situ*.

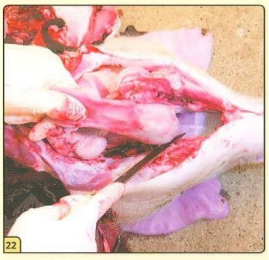


Examine the abdominal viscera *in situ*. Before handling the organs, stop to collect all "clean" tissue samples for microbiology and histopathology. At a minimum, collect samples from lung, liver, spleen, kidney, and lymph nodes, as well as samples of any lesions present.

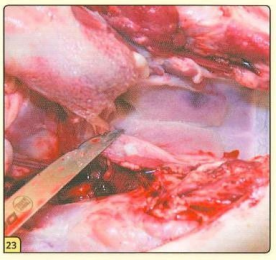


Extend the cut up to the level of the mandibles.

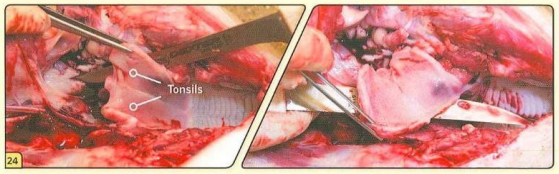
Cut along the medial aspect of both mandibles to free up the tongue.



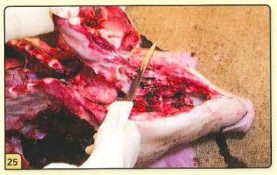
Pull the tongue ventrally and caudally to expose the oral cavity for inspection.



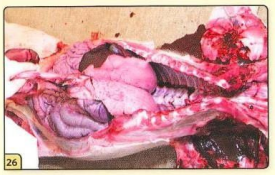
Cut between the hyoid bones to disarticulate the hyoid apparatus.



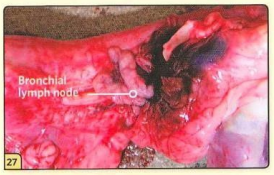
Identify the tonsils, located on the dorsal aspect of the oral cavity, caudal to the hard palate. Remove the tonsils and submit them for microbiology and histopathology.



Retract the tongue and cut the attachments along the trachea and esophagus, working your way down to the level of the thoracic inlet.

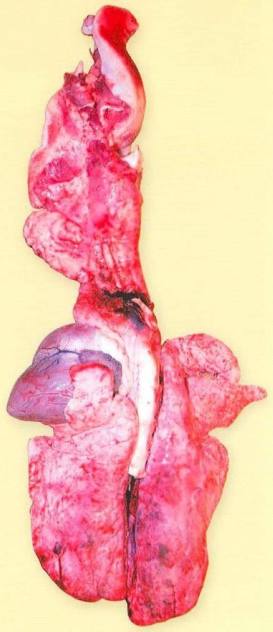


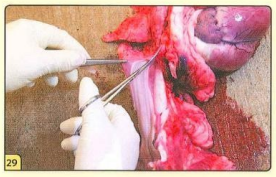
Dissect around the lungs and heart to free up the pluck. Set the pluck aside for a more detailed examination.



Locate and examine the tracheobronchial and mediastinal lymph nodes. The tracheobronchial lymph nodes are located at the bifurcation of the primary bronchi.

Mediastinal lymph nodes are often scattered, but can be found in the cranial mediastinum, associated with the large blood vessels, trachea, and esophagus; in the middle mediastinum, dorsal to the aortic arch; and in the caudal mediastinum, caudal to the aortic arch and ventral to the aorta.

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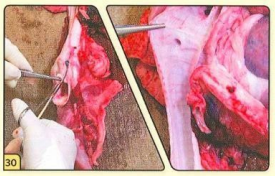
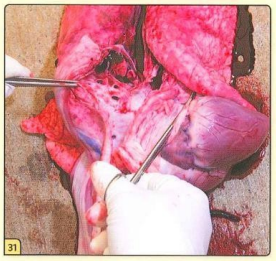
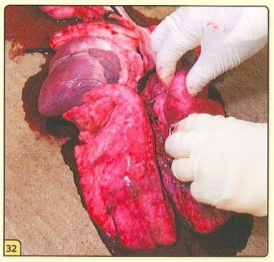
Open and examine the lumen of the esophagus.


30

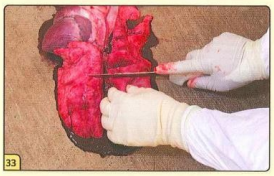
Open and examine the lumen of the trachea.


31

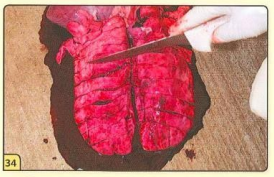
Open and evaluate the large airways of the bronchi.


32

Palpate the entire lung field to assess for any abnormalities.


33

Incise the lungs by making a series of "bread loaf" slices across the entire lung field.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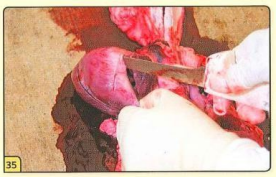
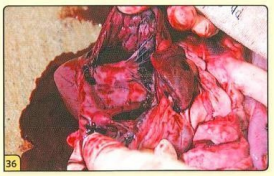
Palpate and examine each slice, assessing for masses and consolidation.


35

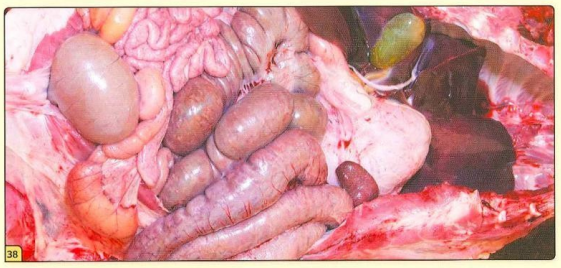
Open the left side of heart by cutting through the free wall of the left atria and left ventricle.


36

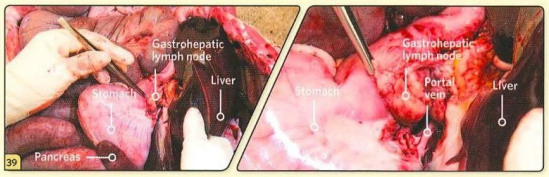
Follow the course of blood flow from atria to ventricle, evaluating the chambers, valves, and myocardial walls.


37

Repeat the process on the right side of the heart.

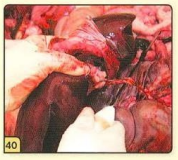

38

Observe abdominal viscera *in situ*.



39

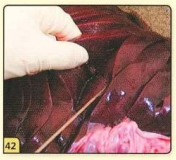
Identify and inspect the gastrohepatic lymph nodes, located between the stomach and liver, adjacent to the portal vein.


40

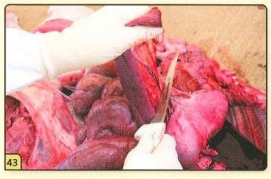
Cut the attachment of the liver and set the liver aside for a more detailed inspection.


41

Make a series of slices across the entire liver.


42

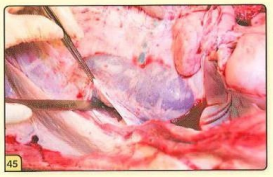
Evaluate each slice, assessing for abnormal areas that require sampling. Collect a representative section of liver for diagnostic testing.


43

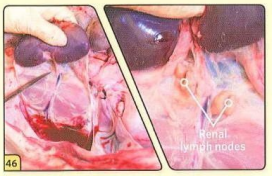
Identify the spleen, located under the stomach. Remove the spleen and set it aside for a detailed inspection.


44

Make a series of slices across the spleen, evaluate the sections, and collect tissue samples.

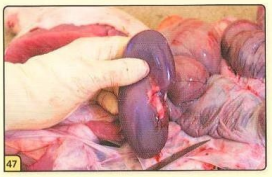

45

Identify the kidneys, located dorsally in the retroperitoneal space.

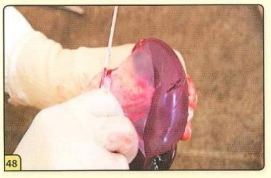


46

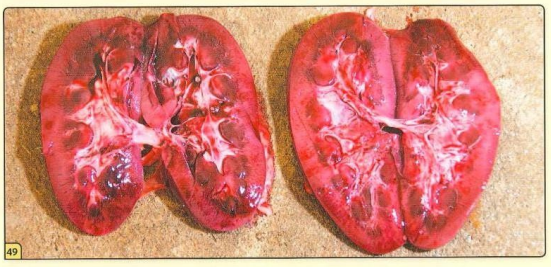
Dissect around the kidneys, and reflect them medially to expose the renal vessels. Identify the two renal lymph nodes on either side of the blood vessels, close to the kidneys.


47

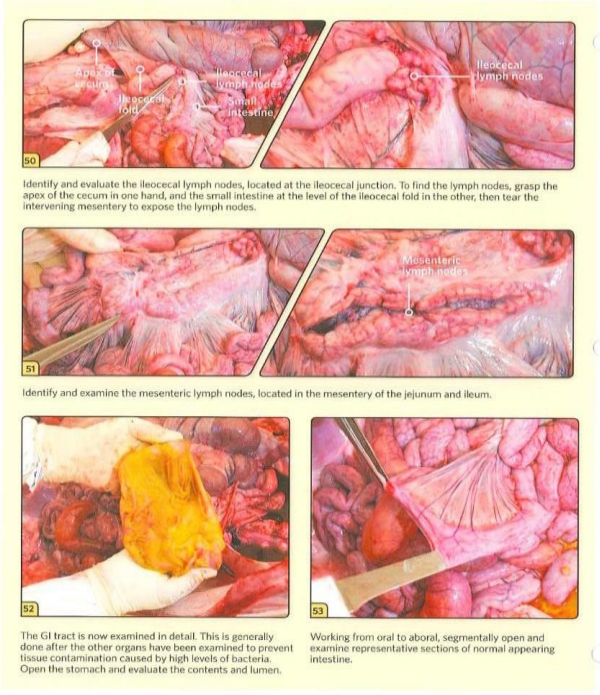
Remove the kidneys.

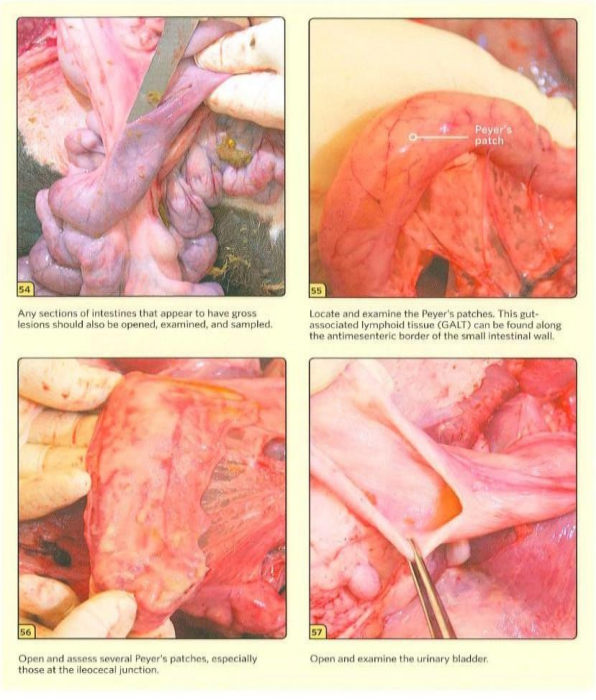

48

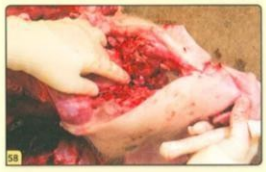
Peel and remove the outer capsule of the kidney.


49

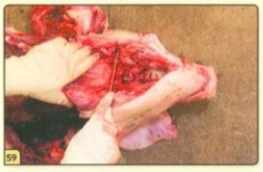
Make a sagittal cut through each kidney. Examine the inner kidneys and collect tissue samples.


Apex of cecum
Ileocecal fold
Ileocecal lymph nodes
Small intestine
Ileocecal lymph nodes
50
Identify and evaluate the ileocecal lymph nodes, located at the ileocecal junction. To find the lymph nodes, grasp the apex of the cecum in one hand, and the small intestine at the level of the ileocecal fold in the other, then tear the intervening mesentery to expose the lymph nodes.
Mesenteric lymph nodes
51
Identify and examine the mesenteric lymph nodes, located in the mesentery of the jejunum and ileum.
52
The GI tract is now examined in detail. This is generally done after the other organs have been examined to prevent tissue contamination caused by high levels of bacteria. Open the stomach and evaluate the contents and lumen.
53
Working from oral to aboral, segmentally open and examine representative sections of normal appearing intestine.

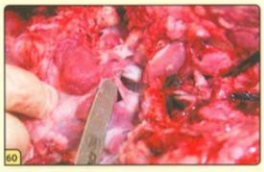

54
Any sections of intestines that appear to have gross lesions should also be opened, examined, and sampled.
Peyer's patch
55
Locate and examine the Peyer's patches. This gut-associated lymphoid tissue (GALT) can be found along the antimesenteric border of the small intestinal wall.
56
Open and assess several Peyer's patches, especially those at the ileocecal junction.
57
Open and examine the urinary bladder.



To remove the head, identify the atlanto-occipital joint by palpation. Flexing and extending the head can aid in identifying the location of the joint.

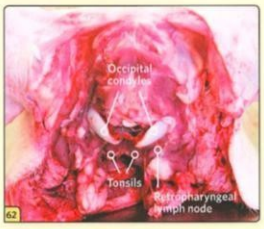


Cut the soft tissues caudal to the atlanto-occipital joint and the ramus of the mandibles.

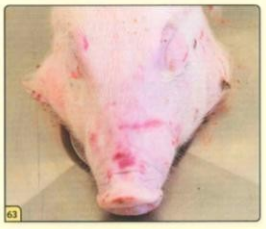


Cut the junction between the 1st cervical vertebrae and the occipital junction.

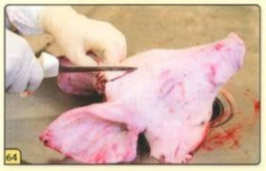
Cut the spinal cord and disarticulate the head.



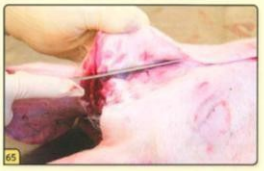
Identify and examine the retropharyngeal lymph nodes, located ventral to the occipital condyles and dorsolateral to the tonsils.



To facilitate opening the skull, the head can be placed on an elevated table.

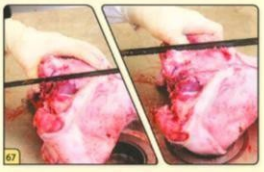


To remove the brain for testing, begin by making a mid-line cut through the skin.

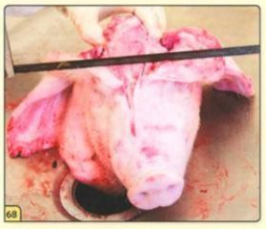


Peel the skin bilaterally to expose the underlying skull bone.

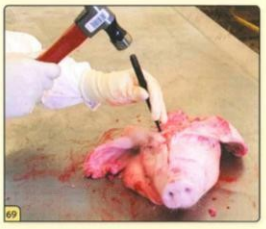
Using the bone saw, make a cut on the medial aspect of both occipital condyles.



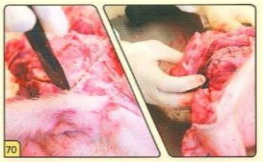
Continue the first two cuts by extending them vertically along the frontal sinuses



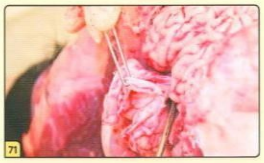
Make a final cut to connect the two vertical cuts along the sinuses.



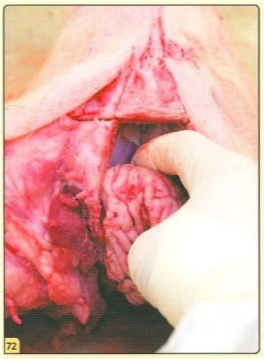
Use a hammer to drive the chisel into the cut made in the skull bone.



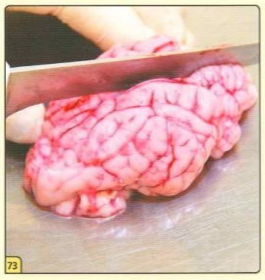
Use the chisel to pry open the skull bone and expose the brain.



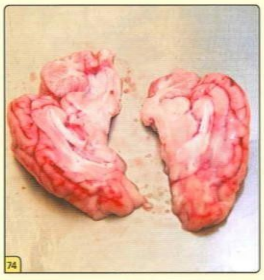
Use forceps and scissors to cut away the dura mater overlying the brain.



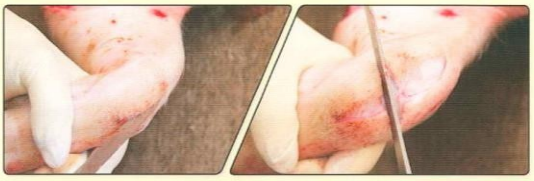
Using your fingers, gently work the brain from the skull. Sever the cranial nerves and remove the brain.

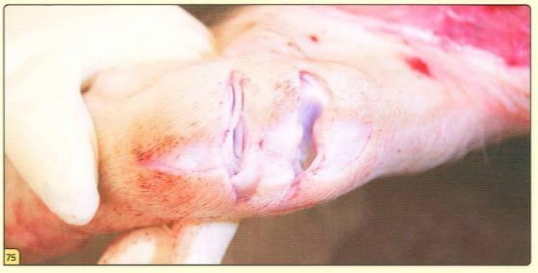


Place the brain on a clean work surface. Make a sagittal cut down the middle of the cerebrum and cerebellum to divide the brain in half.

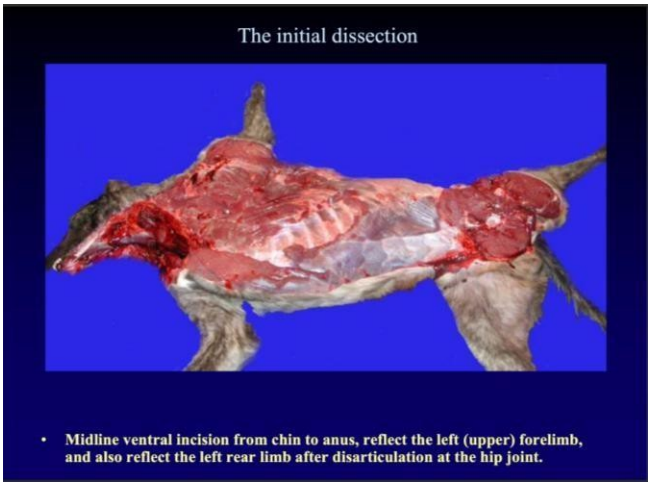


Submit one half as fresh tissue for virology and the other half fixed in formalin for histopathology.

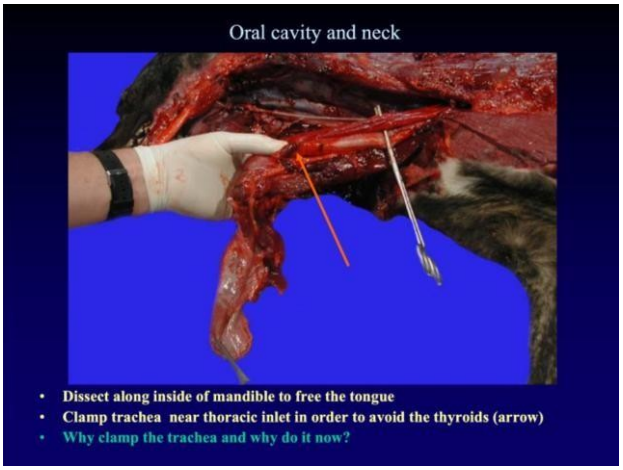


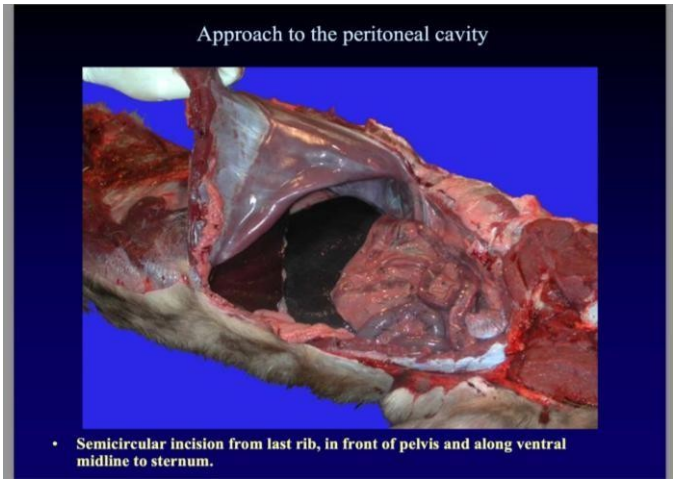


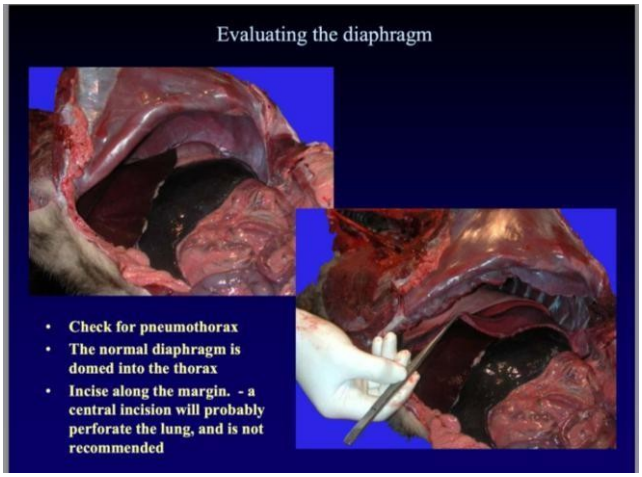
Open several joints, including the carpus and stifle, and examine the joint fluid and cartilage surfaces.

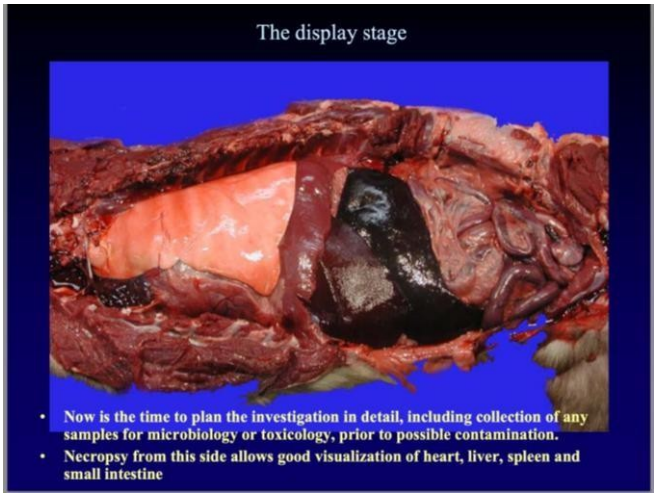


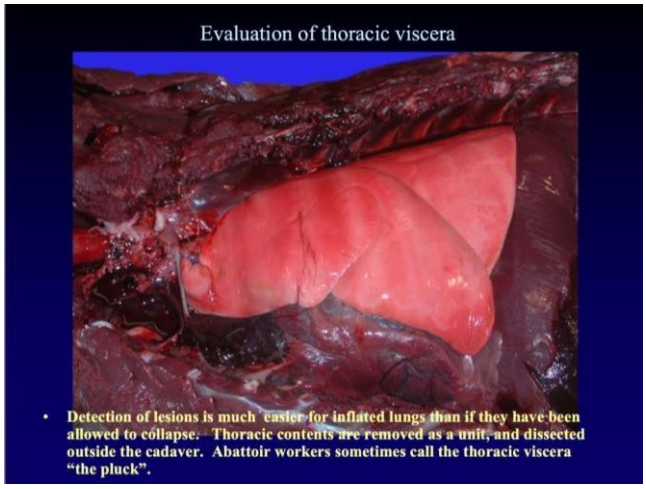
11.3. Postmortem steps for Dogs

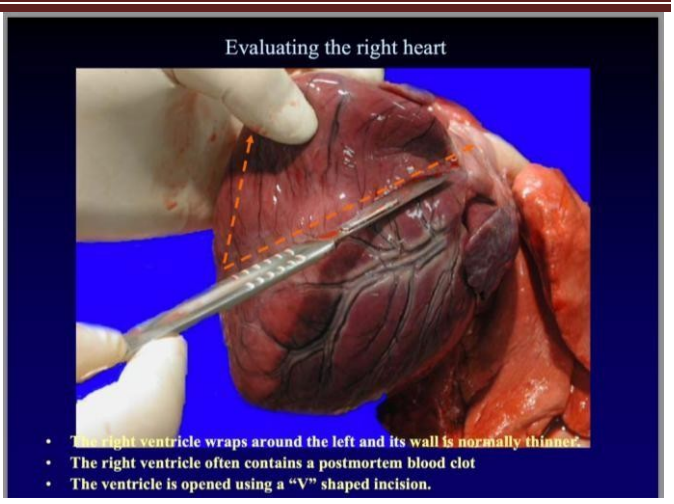


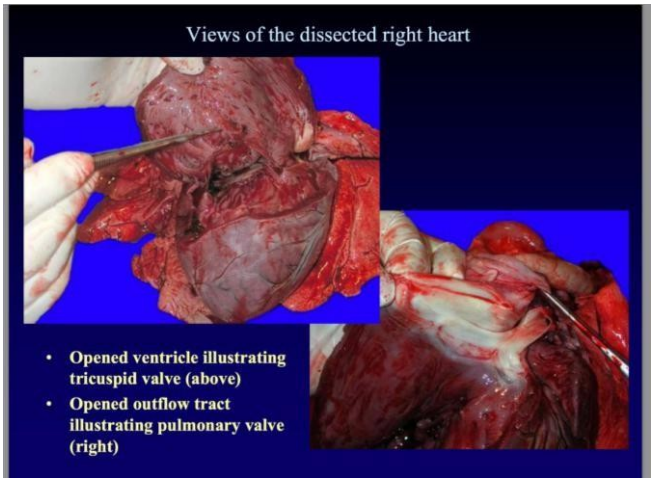


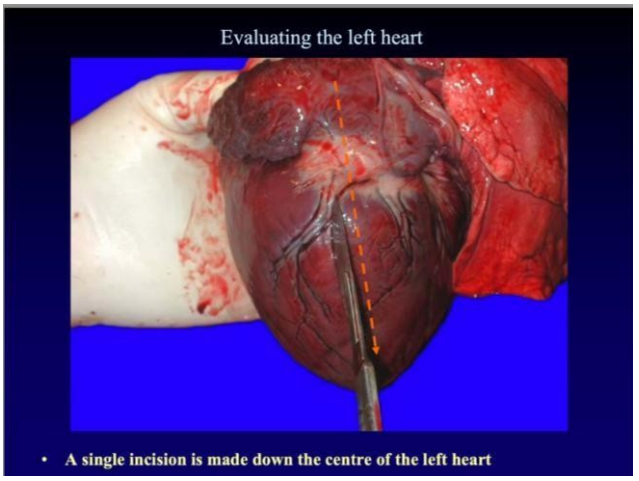


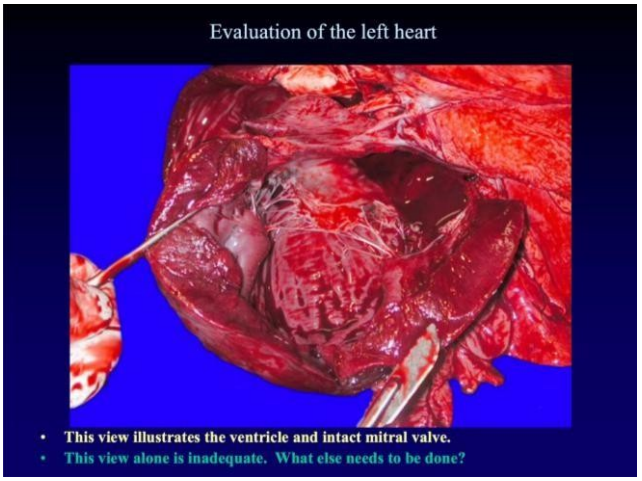


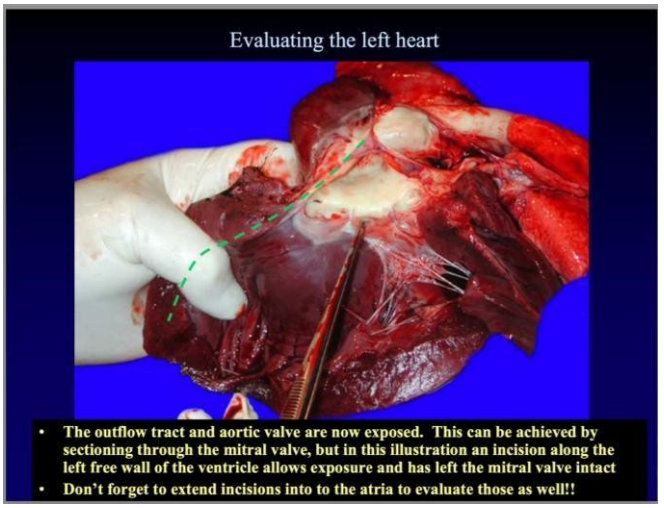

Evaluation of thoracic viscera
• Detection of lesions is much easier for inflated lungs than if they have been allowed to collapse. Thoracic contents are removed as a unit, and dissected outside the cadaver. Abattoir workers sometimes call the thoracic viscera "the pluck".


Evaluating the right heart
• The right ventricle wraps around the left and its wall is normally thinner.
• The right ventricle often contains a postmortem blood clot
• The ventricle is opened using a "V" shaped incision.

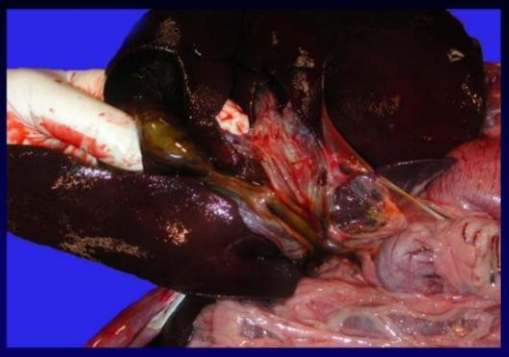

Views of the dissected right heart
• Opened ventricle illustrating tricuspid valve (above)
• Opened outflow tract illustrating pulmonary valve (right)


Evaluating the left heart
• A single incision is made down the centre of the left heart


Evaluation of the left heart
• This view illustrates the ventricle and intact mitral valve.
• This view alone is inadequate. What else needs to be done?


Evaluating the left heart
• The outflow tract and aortic valve are now exposed. This can be achieved by sectioning through the mitral valve, but in this illustration an incision along the left free wall of the ventricle allows exposure and has left the mitral valve intact
• Don't forget to extend incisions into to the atria to evaluate those as well!!

Where to start on the abdominal viscera?

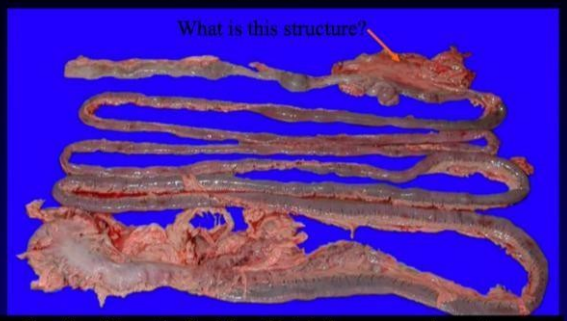


- Check patency of the gall bladder

The spleen

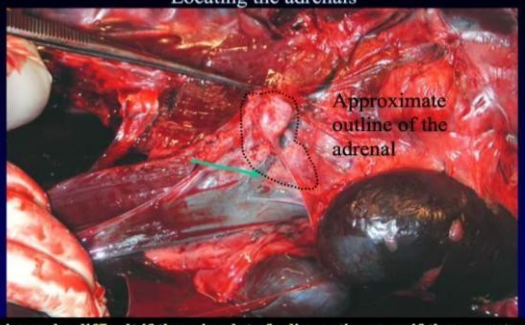
- Dissect the spleen free of its attachments and remove for evaluation
- Spleens in dogs are commonly engorged with blood because of barbiturates

Evaluating the intestinal tract



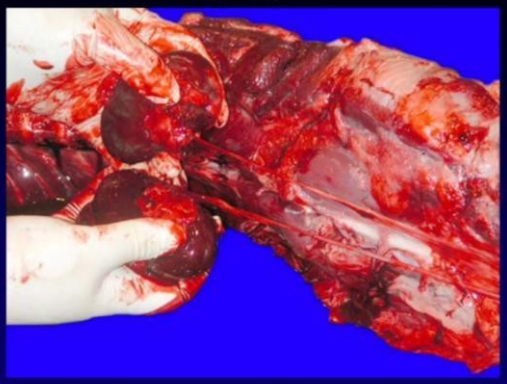
- Dissection of the gut is often left until late in the necropsy unless gastro-intestinal disease is suspected. Why?
- The intestinal tract is removed, checking the mesentery for lesions during this dissection.
- In companion animals we routinely open the entire tract, the stomach best approached by opening along its greater curvature.

Locating the adrenals



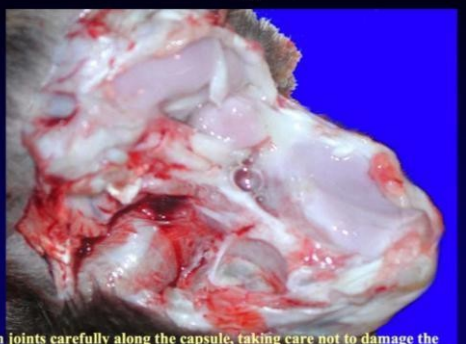
- This can be difficult if there is a lot of adipose tissue, or if they are atrophic
- Best to locate them prior to disturbing the kidneys
- The large vein that runs across the adrenal is distinctive (arrow) and helps distinguish it from nearby lymph nodes, which may also have a similar external color.

The urinary tract

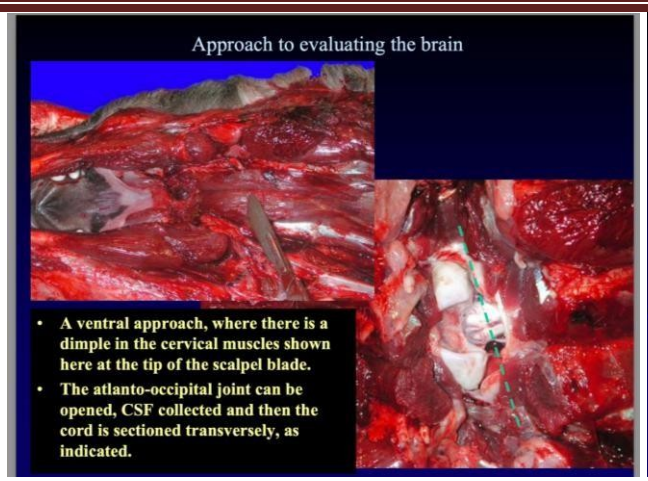


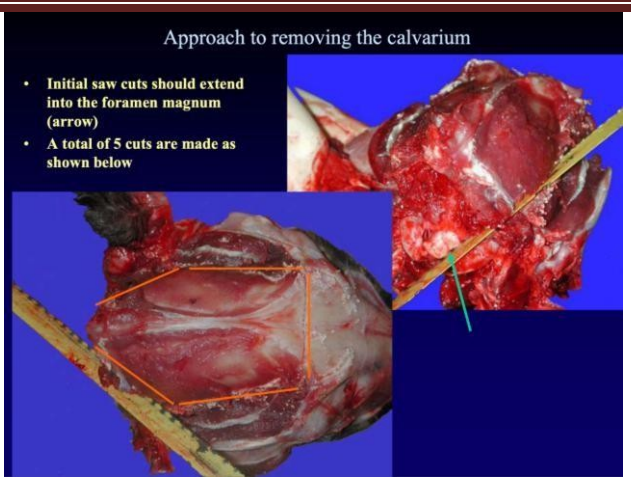
- Dissect kidneys free of vascular attachments but preserve ureters

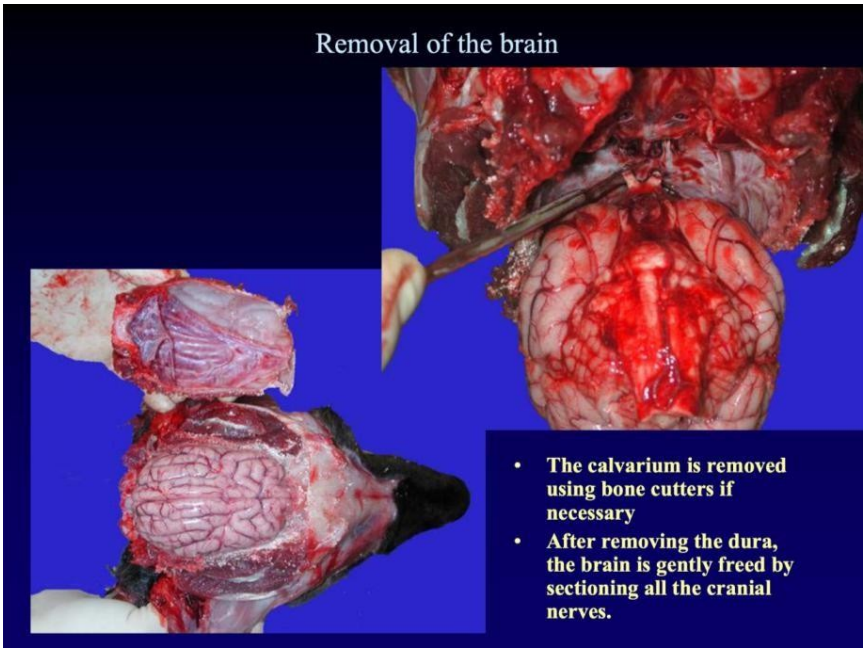
Evaluating joints



- Open joints carefully along the capsule, taking care not to damage the underlying cartilage.
- How many joints should you open before considering sufficient have been checked?
- What is the character of normal joint fluid, and of articular cartilage?


Approach to evaluating the brain
A ventral approach, where there is a dimple in the cervical muscles shown here at the tip of the scalpel blade.
The atlanto-occipital joint can be opened, CSF collected and then the cord is sectioned transversely, as indicated.


Approach to removing the calvarium
Initial saw cuts should extend into the foramen magnum (arrow)
A total of 5 cuts are made as shown below


Removal of the brain
The calvarium is removed using bone cutters if necessary
After removing the dura, the brain is gently freed by sectioning all the cranial nerves.

SOP No: NCAH/LSU/PM 02
Title: SOP on Postmortem Examination in Avian
Version No: 2, Total Pages:6
Issue Month/Effective Date: May 2026
Revision: Summary: Inserted pictures for reference
Supersedes Version No: 1
Prepared by: Postmortem Section, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RLDC, RVH&EC, SVL, DVL

1. **Purpose**
   1.1. To outline the proper procedures for performing a necropsy and post mortem examination in poultry.
   1.2. Necropsy is to be performed for diagnostic purposes, disease outbreak, and research in poultry.

2. **Responsibility**
   2.1. Veterinarians, Veterinary para-professionals / Lab Technicians conduct necropsies and post mortem examinations on cadavers died due to disease or on research birds.
   2.2. Necropsies must be performed by investigators and staff when unexpected morbidities or mortalities are observed in a flock especially in the farms.

3. **Apparatus**
   3.1. Rubber boots, protective clothing, rubber apron or lab coat, rubber gloves.
   3.2. Knives – large and small, with sharpening steel.
   3.3. Scissors (various sizes) and saws; bone cutters
   3.4. A number of sterile, open-mouthed screw top jars of different sizes for specimen collection
   3.5. Sterile swabs in test tubes
   3.6. Alcohol cleaned slides for smears, preferably in a rack or box
   3.7. Spatula
   3.8. Plastic bags for specimens
   3.9. Petri dishes
   3.10. Labels
   3.11. Soap, water, disinfectant and towel.

4. **Reagents, solution and buffer**
   4.1. 10% Formalin or Buffer Neutral Formalin
   4.2. 70% alcohol for wetting and disinfecting the skin,
   4.3. 96-100% ethyl alcohol (for fixing specimens suspected of having gout, and 100mg/1ml for PCR testing),
   4.4. Normal saline (0.85% NaCl) with a pipette (for parasitological examination),

5. **Procedure**

Basic Necropsy Needs

- A flat hard surface in a well-lighted area.
- Access to water and towels.
- Knife or scissors.

Consider gloves and a face mask if we suspect a potentially zoonotic disease (transmissible to humans) as the cause of illness or death.

Performing a Necropsy

5.1. Wet down the feathers with a disinfectant solution to limit the distribution of feathers during the dissection.
5.2. Place the bird on its back with its feet towards you.
5.3. Grasp both legs and push down and away from the pelvis to loosen the joints.
5.4. Tent the skin over the abdomen and cut with scissors or knife.
5.5. Remove the skin overlying the abdomen and breast (from neck to cloaca).
5.6. Examine the breast muscle for decreased muscle mass, paleness (anemia), or bruising.
5.7. Incise the abdominal muscle and cut through the ribs on the sides of the keel bone.
5.8. Grasp the keel near the abdomen and pull upwards to expose the internal organs and chest cavity.
5.9. Examine the liver for changes in size or discoloration, white or yellow spots, abscesses, and/or tumors.
5.10. Examine the air sacs for increased thickness and increased cloudiness. The normal air sac surfaces look like soap bubbles or clear cellophane wrap.
5.11. Cut the gastrointestinal (GI) tract between the esophagus and proventriculus.
5.12. Remove the proventriculus, ventriculus (gizzard), small intestines, large intestine, ceca, and cut off at the level of the cloaca. The pancreas will also be removed. It is the pinkish tan organ cradled within the loop of duodenum (a section of the small intestine).
5.13. Cut all attachments close to the intestines and set the GI tract aside. At the end of the necropsy, these organs can be opened up and examined for internal parasites.
5.14. Next, remove the liver and spleen. A green discoloration of the liver near the gall bladder is a normal finding. The spleen is the reddish, round organ located at the junction of the proventriculus and gizzard.
5.15. Now you can observe the organs located near the backbone of the carcass.
5.16. Examine the kidneys, which are elongated, lobulated organs that are embedded in the backbone of the bird, and the left ovary/oviduct (or paired testes), which are positioned on top of the kidneys.
5.17. The lungs, which are attached to the ribs, can be gently teased out of the ribcage for further examination.
5.18. The outer surface of the heart should be examined for a cloudy, thickened appearance, suggesting pericarditis. Also, note if excessive fluid is located between the heart and the pericardium (membranous covering of the heart).
5.19. Next, turn the bird around to face you and cut through the corner of the beak.
5.20. Extend the cut through the throat and down towards the heart.
5.21. Examine the interior surface of the esophagus and crop. Look for the presence of food and/or parasites (worms) in the crop. If the inside surface appears to resemble a towel, it may be an indication of a fungal infection called “crop mycosis.”
5.22. Next, cut through the larynx, trachea, and syrinx. The inside surface should be free of excess mucus.
5.23. Turn the bird back to the previous positioning — feet in front of you.
5.24. The sciatic nerve located on the interior upper thigh (located under muscle) should be exposed on both legs. The nerves should be the same size bilaterally with no swellings. Enlargement of this nerve can be an indication of Marek’s disease.

5.25. With a sharp knife, cut through the stifle and hock joints, looking for yellow or white pus-like material, blood, or excess fluid. Joints should appear shiny and white with just a small amount of clear, sticky fluid inside.
5.26. To find the bursa of Fabricius, cut through the cloaca and look for a grape-like structure towards the rear of the bird. The older the bird—the smaller the bursa. The bursa diminishes in size as the bird reaches sexual maturity.
5.27. Cut the bursa in half. It should have wrinkles running parallel to each other on the surface and be cream colored in appearance. Note any discoloration or swelling.
5.28. Now return to the GI tract and starting with the proventriculus, cut lengthwise. The inside wall is bumpy and this is normal as these are digestive glands.
5.29. Cut through the ventriculus, intestines, and ceca. Note the appearance of the inside walls (mucosa) and the presence of parasites (worms), blood, and/or a thickened or discolored surfaces.
5.30. Dispose of the carcass properly and disinfect surfaces and tools.

6. Risk assessment

6.1. All carcasses should be handled with care especially if they were known to present risk of zoonotic disease like Avian influenza.
6.2. In such cases, protective clothing, including boots, gloves, face mask and goggles must be worn. The carcasses should be burned or deep buried under stones with quick-lime.
6.3. No eating, drinking, grooming, or other activities that are a means of exposure are permitted in necropsy areas.
6.4. Transport unfixed tissues in leak-proof containers.

7. Waste disposal

7.1. Decontaminate instruments before cleaning them.
7.2. Clean and disinfect all work surfaces or the premises where necropsy was conducted.
7.3. Decontaminate self (e.g., disinfect and remove boots, gloves, and coveralls).

8. References

- University of Minnesota, Veterinary Diagnostic Laboratory, Standard Operating Procedure (2016).
- University of Queensland, School of Veterinary Science, Standard Operating Procedures (2017).
- USDA, Animal and Plant Health Inspection Service, National Veterinary Services Laboratories, Guidelines for Necropsy.
- University of South Florida, Standard Operating Procedure, Necropsy and Post Mortem Examination (2012).
- Western Australian Agriculture Authority. Department of Agriculture & Food. A visual guide to Avian Necropsy (2012).
- Meredith F. D. and Teresa Y. M. 2006.Poultry Necropsy Basics. Thepoultrysite.com

9. Annexure: Steps in necropsy in avian

Department of Agriculture and Food
GOVERNMENT OF WESTERN AUSTRALIA

A visual guide to a chicken necropsy

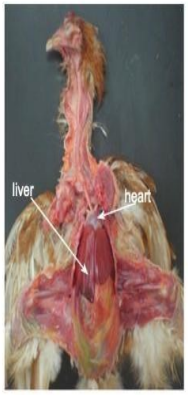
Equipment needed for a chicken necropsy:

- standard post-mortem kit including scalpel, forceps, scissors
- secateurs
- dry swabs, jars for individual fresh samples and pooled formalin fixed tissues
- swabs in media for bacterial culture
- 0.5 mL sterile saline (drip fluid) or viral transport media (VTM).

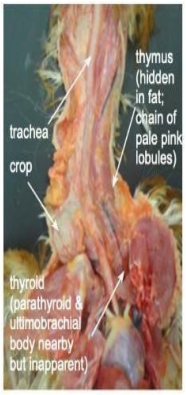
Below is a visual guide to a thorough chicken necropsy. Correct necropsy and sampling will increase the likelihood of a definitive diagnosis. Always include a detailed history and description of lesions on the Animal Health Laboratories (AHL) Submission Form when submitting samples. For more information, contact the AHL on (08) 9368 3351.

Skin and feathers removed displaying:

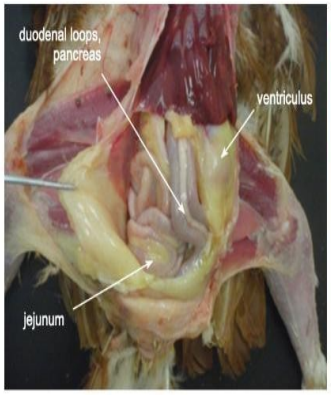
a) coelomic musculature



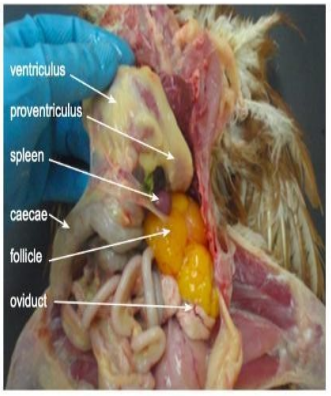
b) neck and cranial coelom



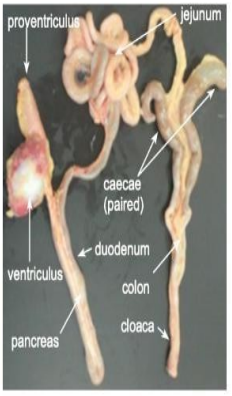
Coelomic cavity



Coelomic cavity with gastrointestinal tract reflected



Gastrointestinal tract anatomy

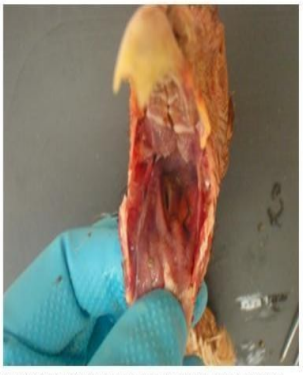


External examination

Use dry swabs to firmly swab the cloaca and trachea mucosa, then snap swab heads into separate viral transport media (VTM) or saline for avian influenza and Newcastle disease virus testing. (VTM is available from AHL or district veterinary officers.)

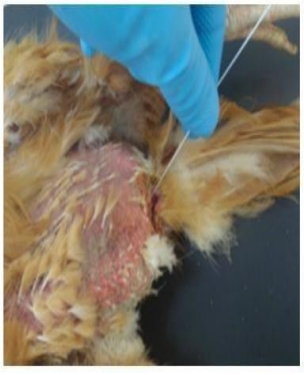
Examine the feathers, skin and limbs. Use sticky tape to collect mites and tape to a microscope slide for identification. Take skin scrapes of crusting lesions to reveal mites.

Collect a tracheal swab



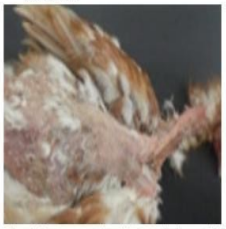
A scissor cut at the corners of the mouth helps expose the trachea.

Collect a cloacal swab



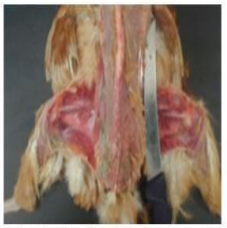
Post-mortem approach

1: Pluck the feathers from the dorsum and ventrum



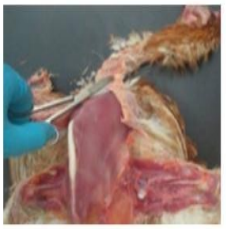
Check for trauma, dog bites and dermatitis.

2: Disarticulate the legs



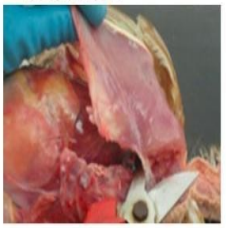
Reflect the legs laterally by incising ventral thigh and disarticulating the femoral head.

3: Expose the pectoral muscle



Reflect or remove the coelomic skin cranially over the sternum and up to the neck to the intermandibular space.

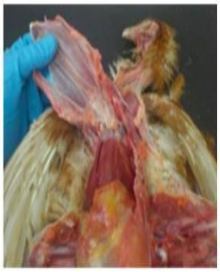
4: Visualise the air sacs while removing keel/ pectoral muscle



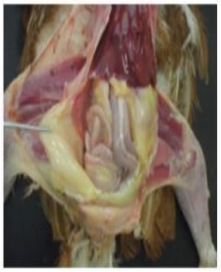
Cut the ribs from the lateral chest and the clavicle and coracoids bones at the thoracic inlet. Lift the keel and pectoral muscles to expose the air sacs. Check the air sacs – they should be clear/cling film like. Opacity in the air sacs may be airsacculitis.

Post-mortem approach *(continued)*

5: Remove the sternum

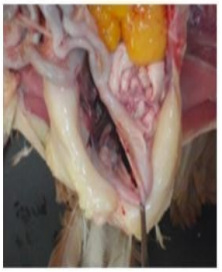


6: Reflect the coelomic musculature



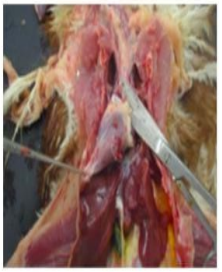
Reveal the gastrointestinal tract. If fibrinous exudate is present in the coelom or air sacs, a swab should be placed into bacterial transport media.

7: Visualise the bursa in dorsal cloacal wall



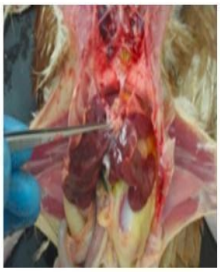
Reflect the ventriculus cranially and to the right. Visualise the bursa/remnant by moving distal colon to the right. The bursa will have regressed in sexually mature birds. Fixed bursa should be taken in younger birds.

8: Remove the heart



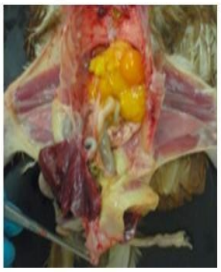
Remove the heart at base (collect a fixed and fresh sample). Examine the dorsal air sacs once the heart is removed.

9: Cut the base of the oesphagus



Sever the oesophagus just cranial to liver. Grasp the oesophagus firmly then reflect liver/gut caudally, cutting the mesentery – this will leave the kidneys and reproductive organs in situ.

10: Retract the gastrointestinal tract to expose the reproductive tract



The spleen is present on the newly exposed, dorsal surface of the ventriculus. Sample the liver and spleen for fresh and fixed samples.

Cut the colon distally. Remove the entire gastrointestinal tract and collect fixed samples of the duodenum and pancreas, jejunum, ileum, colon and caecum.

11: Anatomy after removal of the reproductive tract

If gut content is abnormal (very fluid, reddened etc), collect a fresh sample or swab.

If toxicity is suspected, sample fresh proventricular and ventricular content.

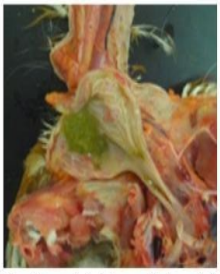
A fresh faecal or distal colon sample can be used to check for enteric parasites like coccidia.

Sample the kidneys (fresh and fixed) and reproductive tract (fixed/fresh ovary and oviduct).

12: Gently peel the lungs from the rib cage

Grasp the caudolateral edge of the lung and apply traction medially. Incise or blunt dissect between the lungs and ribs to remove the lungs. Note the lungs are normally closely moulded to the ribs. Sample the lungs (fresh/fixed).

13: Open the crop and oesophagus



Check for exudates/ plaques that may indicate Trichomonas or Candida infection or hypovitaminosis A.

Check the crop content as a clue to recent food intake.

14: Location of larynx

Examine the upper respiratory tract and alimentary tract.

Examine the larynx and trachea for caseous exudate of infectious laryngeotracheitis (ILT). Sample both fresh and fixed trachea.

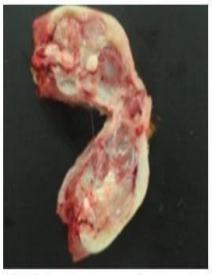
Examine upper respiratory and alimentary tract for plaques (fowl pox).

15: Swab the sinuses for exudates

Remove the head with the secateurs. Cut across the upper beak/nares.

Examine the sinuses for caseous exudate – swab for bacteria and/or Mycoplasma (chronic respiratory disease)

16: Brain exposed for sampling



Skin the head and remove the lower beak. Split the skull to expose the brain by placing a heavy knife or cleaver on the midline.

Swab or shell out half the brain for a fresh sample.

Fix the skull with remaining brain for histopathology.

Remember: Sample lesions if not included in the samples mentioned above; fresh and fixed samples are ideal if lesions are large enough. **More information:** contact the AHL on (08) 9368 3351.

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Prepared by: Postmortem Section, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RLDC, RVH&EC, SVL, DVL, DoFPS

1. **Scope**

To outline the procedures for performing a diagnostic necropsy in fish.

2. **Responsibility**

2.1. Fish health professionals conduct necropsies and post mortem examinations on dead fish that died due to disease.

2.2. Necropsies must be performed by investigators and staff when unexpected morbidities or mortalities are observed in a fish pond.

3. **Apparatus**

3.1. Rubber boots, protective clothing, rubber apron or lab coat, rubber gloves.
3.2. Knives – small, with sharpening steel.
3.3. Scissors (various sizes) and saws; bone cutters
3.4. A number of sterile, open-mouthed screw top jars of different sizes for specimen collection
3.5. Sterile swabs in test tubes
3.6. Alcohol cleaned slides for smears, preferably in a rack or box
3.7. Spatula
3.8. Plastic bags for specimens
3.9. Petri dishes
3.10. Labels
3.11. Soap, water, disinfectant and towel
3.12. Scalpel blade with handle

4. **Reagents, solution and buffer**

4.1. 10% Formalin or Buffer Neutral Formalin
4.2. 70% alcohol for wetting and disinfecting the skin,
4.3. 96-100% ethyl alcohol (for fixing specimens suspected of having gout, and 100mg/1ml for PCR testing),
4.4. Normal saline (0.85% NaCl) with a pipette (for parasitological examination),

5. **Procedure**

5.1. **Visual Assessment** of condition of each fish to observe for an ulcer, reddening underneath the operculum or of junction between gills and body or tissue necrosis is detected

5.2. If necropsy is unable to be performed immediately, **place fish into a plastic bag in an ice slurry**. This is to slow autolytic changes if there is a slight delay in the necropsy.

5.3. **Morphometric Measurements** of fish to include fork length, width, height and weight of individual fish. Note any physical malformations

5.4. **Gill Tissue:** Dissect out 1st gill arch and prepare gill scrape and view under a compound microscope. Dissect out 2nd gill arch and initially view under dissecting scope then prepare a slide using a section of this material and view under compound microscope. These methods will detect the presence of hyperplasia, epitheliocystis, gill fluke or blood fluke eggs.

5.5. **Skin Scraping:** Use the back of a scalpel blade to remove a section of mucus from the skin especially near skin lesions. Place onto a microscope slide with a cover slip and a drop of water, and observe under compound microscope. Look for the movement of live skin parasites.

5.6. **Sampling Organs for Histology:** Dissect out each organ including tissue from the heart, liver, spleen, gonads, kidney, muscle, stomach, hindgut and brain. Look for obvious abnormalities. Take weights of the liver and gonads (if mature) and visually assess their condition.

5.7. **Histology Examination:** Preserve tissues extracted from **Step f** in 10% neutrally buffered formalin and send preserved tissues for histological analyses and subsequent report.

5.8. **Examine Records of Water Quality and Husbandry Activities:** Fluctuating water quality parameters and handling may be a trigger for mortality, especially if there are underlying factors such as high bacterial loads in the culture water.

6. Risk assessment

Fish carcasses should be handled with care especially if they were known to present risk of zoonotic diseases like *Mycobacterium, Erysipelothrix, Campylobacter, Aeromonas.*

7. Waste disposal

7.1. Decontaminate instruments before cleaning them.
7.2. Clean and disinfect all work surfaces or the premises where necropsy was conducted.
7.3. Dispose the carcass as per the waste management guidelines.

8. References

Curtin University, standard operating procedures. (2015). Fish dissection and biopsy collection.

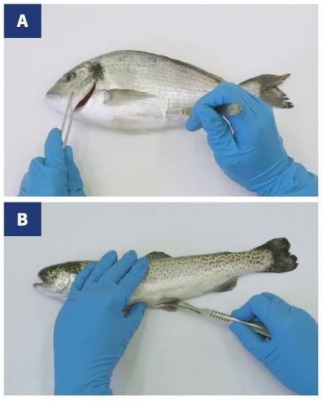
9. Annexure: Postmortem steps for Fish

STEP 1

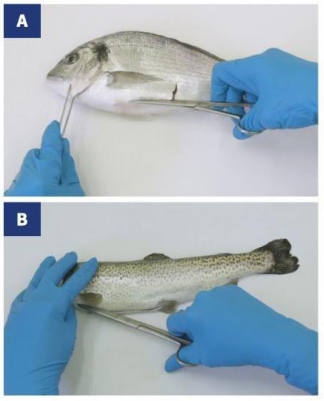
1. Place the fish on its right flank and using a sterile scalpel (new or dipped in alcohol or bleach), make an incision in the abdominal area (Picture 13), avoiding perforation of the digestive system.

2. Carry the incision forward to the throat (Picture 14) and later backward to the anus using a pair of sterile scissors (take care not to cut the gut on the inside).

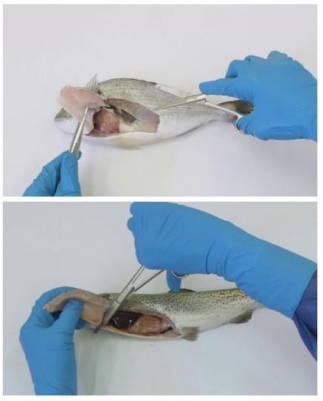
3. Carefully cut away the musculature overlaying the internal organs to expose the intestine (picture 15).



Picture 13: Pelvic incision in seabream (A) and trout (B)



Picture 14: Incision forward to the throat in seabream (A) and trout (B)

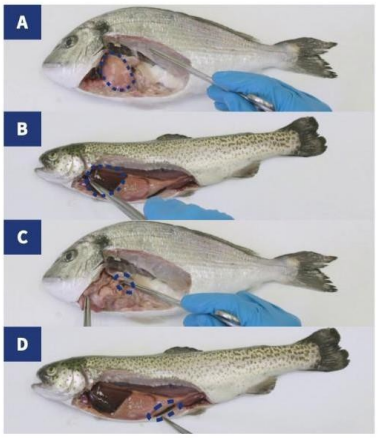


Picture 15: Expose the abdominal cavity

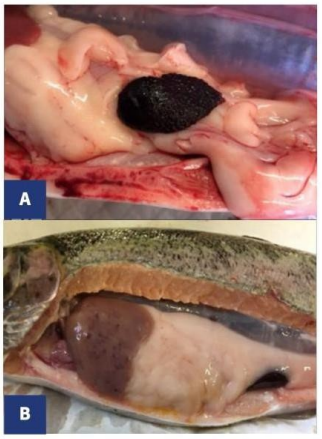
STEP 2

1. Look over the surface of the gut and the muscle flanks, checking for any abnormalities (haemorrhages, ulcers, changes in colour etc.) and note the position of any. You will be able to see vaccine residues and adhesions (if present) if the fish has already been vaccinated

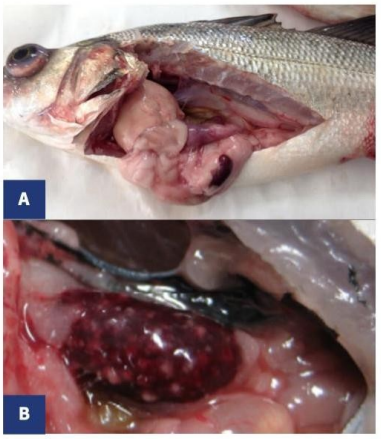
2. Locate the main organs (picture 16): Check and record any changes in colour and/or size/shape of the liver, spleen and gut (pictures 17 and 18).



Picture 16: . A) Liver position in seabream B) Liver position in trout. C) Spleen position in seabream. D) Spleen position in trout.



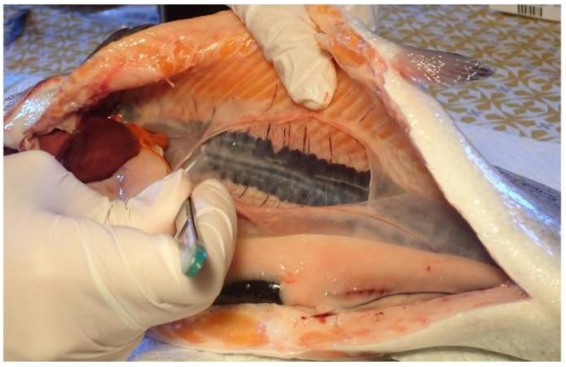
Picture 17: A) Granulated and enlarged spleen in Arctic char due to atypical Aeromoniasis. B) Changes in liver colour and presence of petechiae (red spots caused by minor bleed), pale heart, ascites, enlarged spleen and bile coming from an enlarged gallbladder (typical of anorexia) (Lactococcus garivеae outbreak)



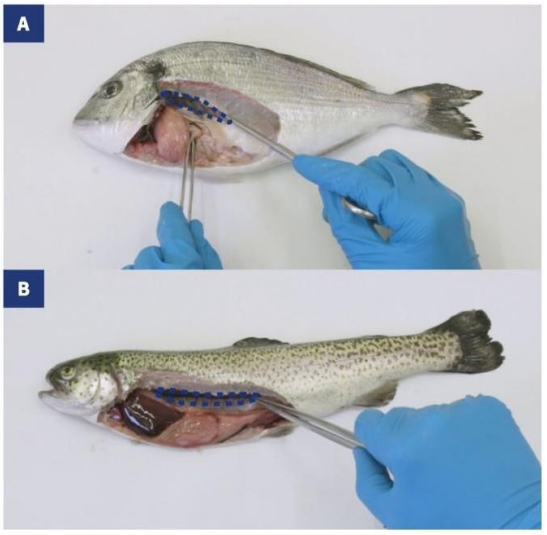
Picture 18: A) Pale liver and enteritis in seabass. B) Nodules on enlarged spleen in seabass (Photobacteria damselae)

STEP 3

1. Then move the alimentary tract to one side until the kidney is exposed, covered by the swim bladder (pictures 19 and 20). Also check and record any abnormality in colour, shape and consistency of the kidney. This is the best time to take bacteriology samples.



Picture 19: Peeling back the swim bladder in large trout and exposing the kidney.



Picture 20: A) Kidney position in seabream B) Kidney position in trout.

! Change gloves, or at least rub them with alcohol, each time a new fish is internally inspected.

SOP No: NCAH/LSU/PM 04
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Supersedes Version No: 1
Prepared by: Postmortem Section, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RLDC, RVH&EC, SVL, DVL, DoFPS

1. **Purpose**

To outline the procedures for performing a necropsy in reptiles.

2. Responsibility

2.1. Wildlife veterinarians conduct necropsies and post mortem examinations on dead reptiles died due to diseases.

2.2. Necropsies must be performed by investigators and staff when unexpected morbidities or mortalities are observed in a reptile population.

3. Apparatus

3.1. Rubber boots, protective clothing, rubber apron or lab coat, rubber gloves.
3.2. Knives – Big & small, with sharpening steel.
3.3. Scissors (various sizes) and saws; bone cutters
3.4. A number of sterile, open-mouthed screw top jars of different sizes for specimen collection
3.5. Sterile swabs in test tubes
3.6. Alcohol cleaned slides for smears, preferably in a rack or box
3.7. Spatula
3.8. Plastic bags for specimens
3.9. Petri dishes
3.10. Labels
3.11. Soap, water, disinfectant and towel
3.12. Scalpel blade with handle

4. Reagents, solution and buffer (refer annexure-ii)

4.1. 10% Formalin or Buffer Neutral Formalin
4.2. 70% alcohol for wetting and disinfecting the skin,
4.3. 96-100% ethyl alcohol (for fixing specimens suspected of having gout, and 100mg/1ml for PCR testing),
4.4. Normal saline (0.85% NaCl) with a pipette (for parasitological examination),

5. Procedure

5.1.External Examination

Before you make any incisions, examine the exterior of the animal and document any irregularities. Use a checklist incorporating the following elements to ensure your examination is complete:

5.1.1. **Skin**: look for scars, irregular pigmentation, and wounds.
5.1.2. **Extremities**: check for regenerated tail or missing limbs and digits.
5.1.3. **Ectoparasites**: mites and ticks often hide in compact areas like the corners of the mouth, in and around the cloaca, the tympanic membrane, and the joints of the legs.
5.1.4. **Muscle condition:**
5.1.5. **Eyes**: a sunken eye is a likely sign of severe dehydration or malnourishment.
5.1.6. **Oral Cavity**: look for any signs of disease or inflammation. In reptiles, an infection (abscess) might appear like cottage cheese or curds, unlike any pus or liquid substance

you would typically find in similar infections in mammals. Inspect the teeth, trachea, and tongue. Abnormal fluid or material found on the roof of the mouth, or choanal slit, might be an indicator of an airway infection. Collect any lesions with adjacent normal tissue.

5.1.7. **Skeletal structure**: the specimen should be carefully palpated for evidence of trauma, fractures, or other anomalies.

5.2. Internal Examination

5.2.1. The animal should be placed flat on their backs.

5.2.2. Using a suitable cutting instrument (scalpel, scissors, knife), make an incision along the ventral midline, or belly, from the anal region/cloaca to just under the lower jaw. A large vein, the ventral abdominal vein, is directly beneath the ventral midline. Shift the incision slightly to the left or right away from this vein to avoid excessive blood spill.

5.2.3. Turn back the skin and muscles on each side while cutting to reveal the body cavity.

5.2.4. When you reach the rib cage, cut through the cartilage on one side of the sternum and pull back the ribs to see the heart and liver.

5.2.5. Fat pads: The size and condition of a reptile's fat pads can be useful indicators of the body condition and diet of the animal (a large fat pad indicates the animal is getting a sufficient amount of food). Usually, they are found in the body cavity, and some fat bodies may also be present near the heart.

5.2.6. Heart: Carefully remove this membrane to observe the three chambers.

5.2.7. Liver: A relatively large, lobed, mahogany color or light brown organ just under the heart.

5.2.8. Gallbladder: The gall bladder can usually be found near the pancreas and spleen, or, in some species, inside the liver. This organ contains bile that gives it a color in between green and yellow.

5.2.9. Lungs: They should be palpated carefully to feel for any nodules or abnormalities. In addition, a small incision should be made to see if there are any parasites or exudate

5.2.10. Digestive system: Examine the gut contents either by cutting open the digestive tract or by simply squeezing the contents out of the tract and into a collection container through either the esophagus or the large intestine.

5.2.11. Urinary genital system: Examine for abnormalities in kidneys and reproductive organs.

6. Risk assessment

Crocodilian carcasses should be handled with care especially if they were known to present risk of zoonotic diseases like *Campylobacter, Leptospira etc.*

7. Waste disposal

7.1. Immediately after the completion of the necropsy, put the animal in a plastic bag for storage and freeze or refrigerate it until it can be properly disposed of in a manner that is compliant with waste management guidelines.

7.2. The table, clothing, and equipment used should be first washed in hot water and detergent, then disinfected with a cleaning agent such as Virkon or phenol.

7.3. All disposable items used (scalpel blades, needles, and latex gloves) should be discarded appropriately in a biohazard or sharps container.

8. References

University of Florida (2016). Necropsies of Reptiles: Recommendations and techniques for examining invasive species.

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Prepared by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RLDC, RVH&EC, SVL, DVL, DoFPS

1. Scope

To conduct a systematic post-mortem examination of elephants to assess cause of death, disease conditions, and pathological changes, contributing to wildlife health monitoring and management.

2. Objective

To determine the cause of death and identify any disease or abnormalities in elephants through thorough post-mortem examination.

3. Responsibility

3.1. Wildlife veterinarians conduct necropsies and post mortem examinations on dead reptiles died due to diseases.

3.2. Necropsies must be performed by investigators and staff when unexpected morbidities or mortalities are observed in a reptile population.

4. Apparatus

4.1. At least 6 quality large necropsy knives, knife sharpener, steel, and/or stone
4.2. Standard large animal necropsy instruments. Multiple scalpel handles, duplicates or triplicates of other instruments. Extra box of scalpel blades, knife sharpener, and a continual supply of sharp knives.
4.3. Sterile instruments for culture collection.
4.4. Containers for sample collection. Cylindrical plastic tubes.
4.5. Culture swabs, sterile urine cups, glass slides.
4.6. Serum tubes for blood and urine collection.
4.7. Aluminum foil and plastic bags for freezing tissues. Whirl-paks of various sizes work well.
4.8. Labels and waterproof marking pens.
4.9. Scale for obtaining organ weights.
4.10. Tape measure (metric), at least 2 meters long.
4.11. Chain saw, axe, or reciprocating saw to cut through the cranium.
4.12. Hammers, chisels and handsaws.
4.13. Small hand meat hooks x 6
4.14. Hoist/crane/small tractor
4.15. Heavy straps, chains, ropes
4.16. Carts on rollers to move heavy parts.
4.17. Coveralls, boots, gloves, caps, masks, protective eye and head gear, face shields
4.18. Waterproof disposable suits are ideal
4.19. Accessible water supply with hose.
4.20. First aid kit.
4.21. Torches
4.22. Ropes
4.23. Magnifying glass
4.24. Crowbar

5. Reagents, solution and buffer

1. 10% Formalin or Buffer Neutral Formalin
2. 70% alcohol for wetting and disinfecting the skin,

3. 96-100% ethyl alcohol (for fixing specimens suspected of having gout, and 100mg/1ml for PCR testing),
4. Normal saline (0.85% NaCl) with a pipette (for parasitological examination)

6. Procedure

Assigning specific tasks to team members ensures an orderly necropsy. Teams can be designated for areas such as the head, forelegs, hind legs, and abdomen, while one person oversees sample collection, labeling, and communication. The head is best dissected after removal from the body. Accessing the brain requires cutting through the thick cranium using tools like a chain saw, axe, or chisel, though a battery-operated reciprocating saw may be safer. A posterior approach involves making three deep cuts at the base of the skull and removing the bone in sections. As bone cutting can produce hazardous fragments, appropriate protective gear must be worn.

Step-wise procedure

6.1. **Positioning**

a. Perform necropsy with the elephant in **left lateral recumbency**.
b. Necropsy follows the **same principles as in other mammals**.

6.2. **External Examination**

a. Assess **body condition** and visible lesions.
b. Examine the **oral cavity** for any abnormalities.

6.3. **Initial Incision**

a. Procedure performed by **at least two persons**.
b. Make a **ventral midline incision**.
c. One person **holds the retractor**, the other **cuts the tensed skin**.

6.4. **Reflection of Limbs and Skin**

a. Reflect **forelegs and hindlegs** by cutting through joints (including hip joint).
b. Reflect the **skin and abdominal muscles** along the midline.

6.5. **Opening the Thoracic Cavity**

a. Extend the incision **cranially up to the tongue**.
b. Expose the **sternum**.
c. Separate ribs at **cartilaginous attachments**.
d. Apply **retractors** to open the thoracic cavity.
e. Reflect ribs away from the carcass.

6.6. **Exposure of Cavities**

a. Expose **thoracic and abdominal organs**.
b. Note: **Pleura are normally adhered (minimal pleural space)**.

6.7. **Organ Removal and Examination**

a. Proceed from **cranial to caudal direction**.
b. Remove and examine the **"pluck" (tongue, trachea, oesophagus, lungs, heart)** en bloc with diaphragm.
c. Examine **liver, stomach, intestines, and kidneys** sequentially.

6.8. **Brain Examination**

- Extract and examine the **brain through the foramen magnum**.

7. Risk Assessment

Some diseases are zoonotic like *Mycobacterium tuberculosis* and *Mycobacterium bovis and one* should always be ruled out during the necropsy and tested for appropriately.

8. Waste Management

The carcass should be buried underground with at least 1 meter of soil covering above it. Bleaching powder should be applied over the carcass before final covering.

9. References

EAZA Elephant Best Practice Guidelines 2020

Mick Millar. 2017.*How to postmortem an elephant. Vet Times*. https://www.vettimes.co.uk

10. Annexure: Postmortem steps for Elephants

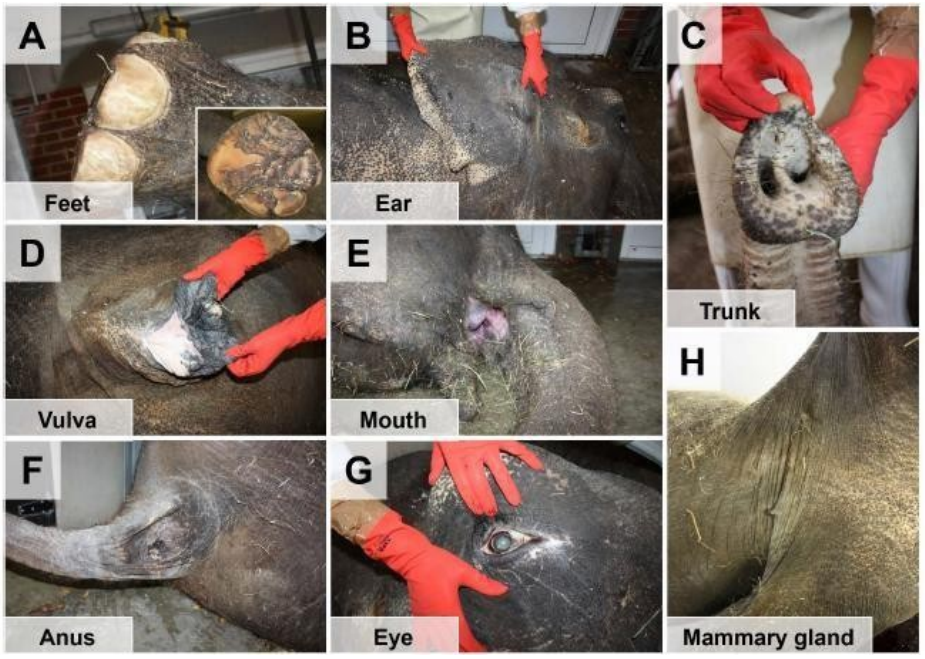

Fig 2. External examination of an aged female Indian elephant. Feet **(A)**, ears **(B)**, trunk (**C**, note the presence of a single -two in African elephants- "finger-like" extension dorsal of the nostrils), vulva (**D**, note the large distance to the anus), mouth **(E)**, anus **(F)**, eyes (**G**) and mammary gland (**H**, located between the front legs).

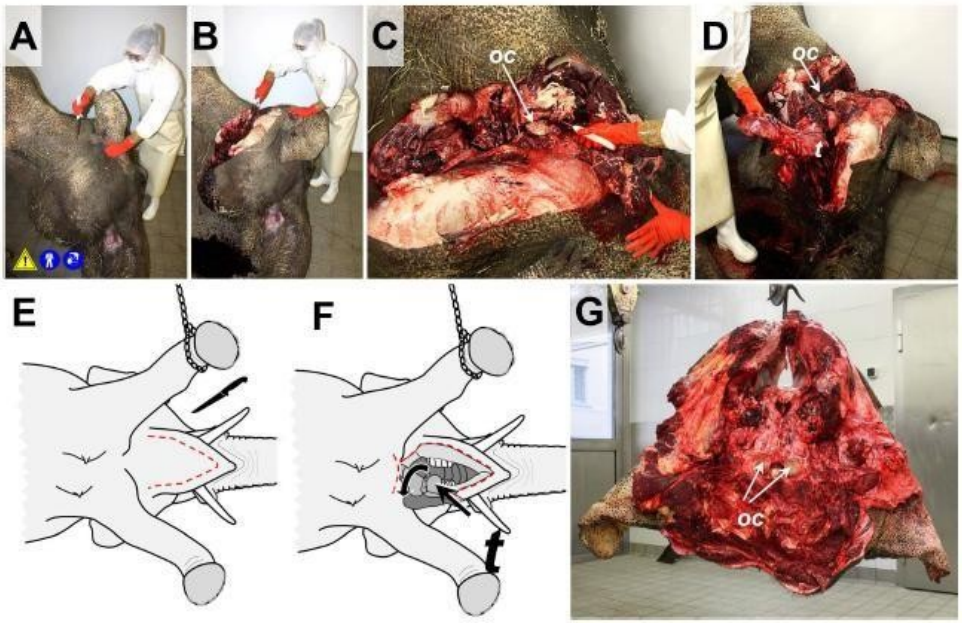

Fig 4. Removal of the head. (A-D) The head is disarticulated in the atlanto-occipital joint. The occipital condyles (*oc*) are indicated. **(D-F)** Tongue **(*t*)**, pharynx and neck organs (larynx, trachea, esophagus, adjacent nerves and vessels, the thyroid and parathyroid glands, and cervical lymph nodes) are removed from the head (but left attached to the rump), as schematically illustrated in **(E-F)**: the *red dotted line* indicates the incision line at the ventral side of the lower jaw. Skin and mouth base are cut through following the medial contours of the lower jaw. The tongue and the adjacent neck organs are mobilized, severing the dorsal and lateral pharynx walls, and flipped backwards. **(G)** Caudal aspect of the removed head (hung up at the lower jaw). Note the occipital condyles (*arrows*) and removed neck organs.

Fig 5. Removal of the forelimb with the scapula (A) and the hindlimb (B) after disarticulation of the coxofemoral joint (C). (D) Elephant body after removal of the right limbs.

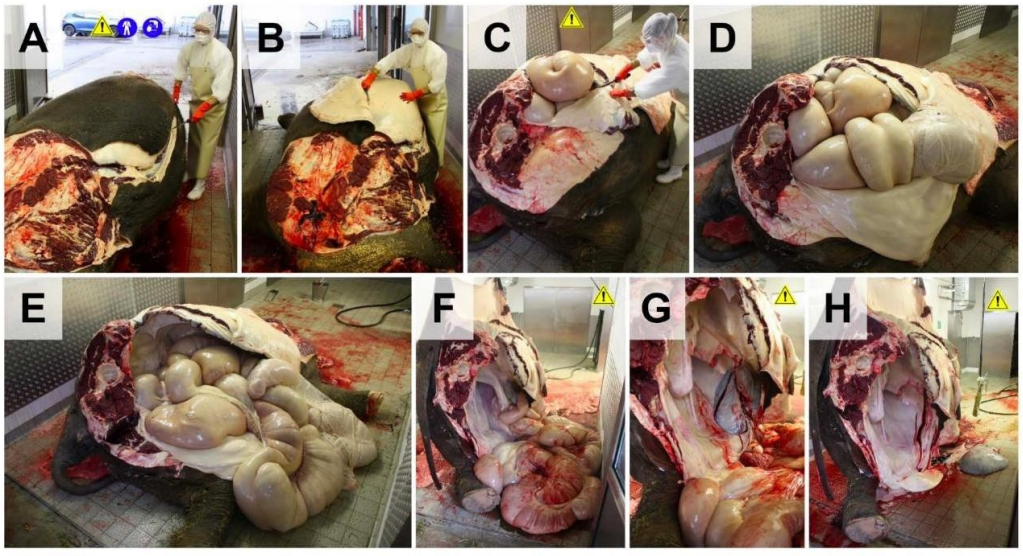

Fig 6. Evisceration of abdominal organs. (A, B) The skin is removed from the lateral abdomen. **(C-E)**. Removal of the lateral abdominal wall. Note that intestines may contain large amounts of gas under pressure. **(F)** For removal of the intestines, the body may be lifted (workplace safety). **(G, H)** Intestines, stomach, spleen, and liver are removed. The urogenital organs, adrenals and large abdominal vessels are removed subsequently, or after removal of the contralateral hind limb.

https://doi.org/10.1371/journal.pone.0338783.g006

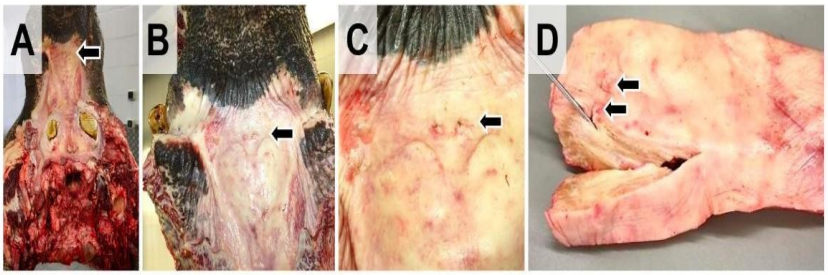

Fig 9. Dissection of Jacobson's organ. (A-C) The bilateral openings of the vomeronasal organ are visible on the surface of the rostral oral mucosa of the maxillary bone (*arrows*). **(D)** Excised tissue sample from the roof of the mouth. *Arrows* mark the oral openings of the Jacobson's organ. One canal of the organ is longitudinally opened and inserted with a probe.

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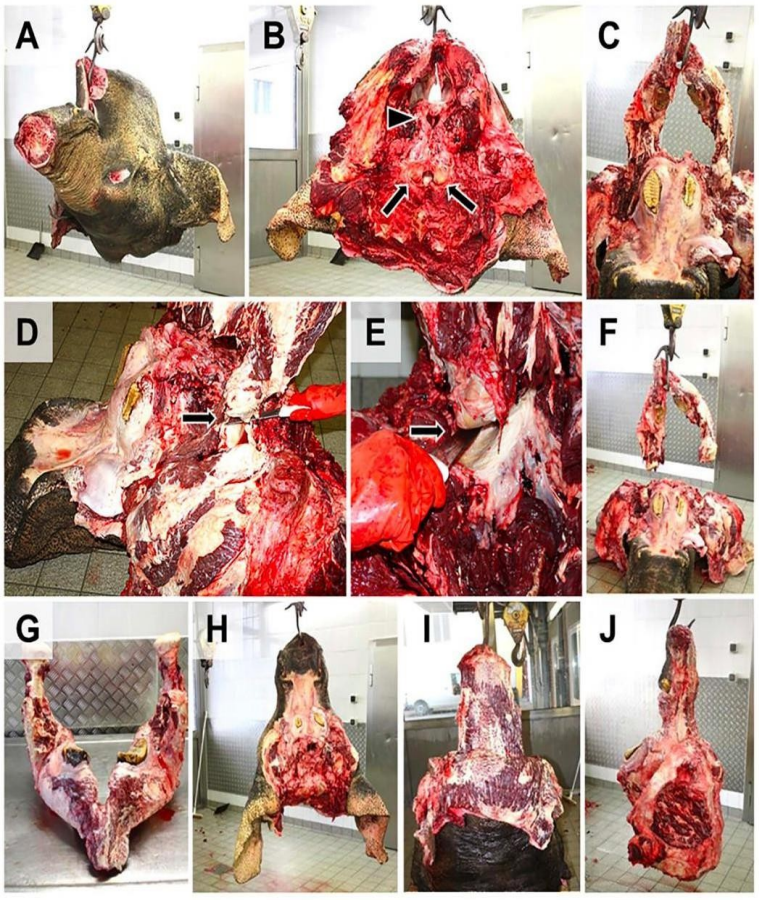

Fig 10. Dissection of the head (part one). (A) Head after removal of eyes and trunk. **(B)** Caudo-ventral aspect of the head. *Arrows* mark the condyles. The *black arrowhead* indicates the section profile of the ventral nasal meatus. **(C-E)** Removal of the lower jaw. The soft tissue of the cheeks is removed from the jaws **(C)** to access **(D)** and sever **(E)** the temporomandibular joint (*black arrow*). **(F, G)** Removed lower jaw. **(H)** Ventral aspect of the head after removal of the lower jaw. **(I, J)** Removal of skin and musculature from the head.

https://doi.org/10.1371/journal.pone.0338783.g010

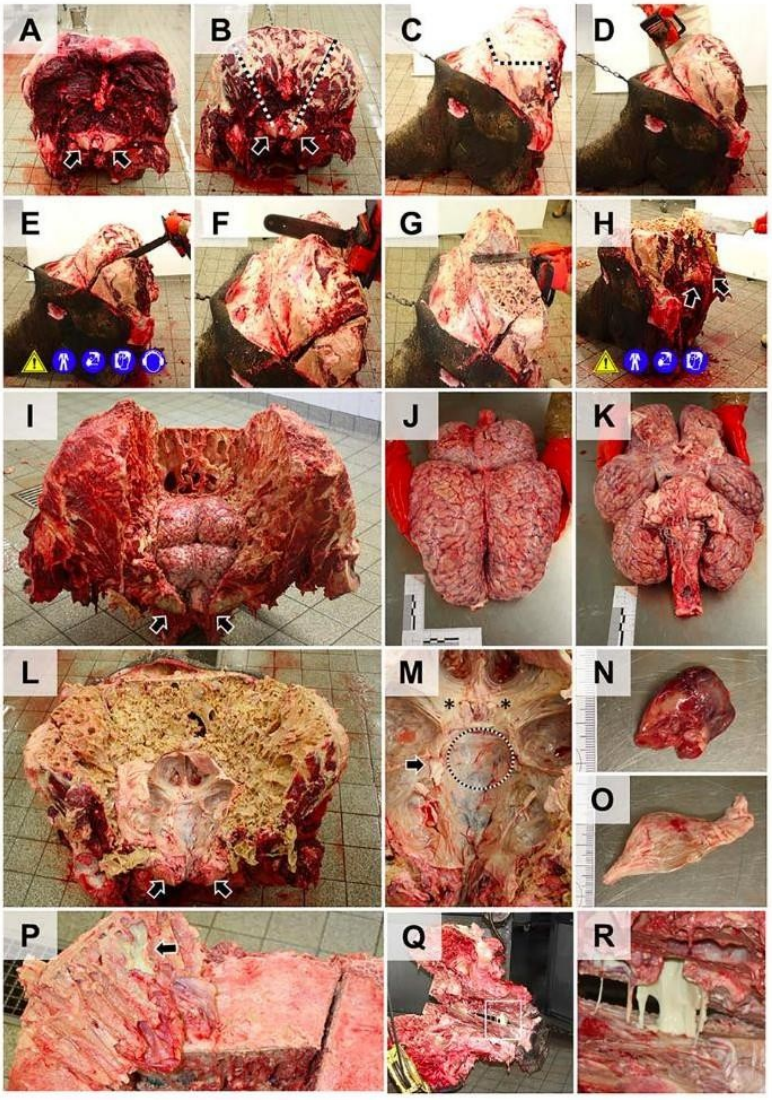

Fig 12. Dissection of the head (part two): Removal of the brain (compare to Fig 11). (A) Caudal aspect of the head. Note that in the present example, the trunk and the lower jaw have not been removed in advance. *Arrows* mark the condyles. **(B)** Removal of neck musculature attached to the occipital bone. **(C)** Lateral aspect. The *dotted lines* in **(B)** and **(C)** indicate the incision lines for removal of the skullcap. **(D-F)** Incision into the frontal sinus. Here a chainsaw is used (workplace safety). **(G)** Removal of a large portion of the dorsal skullcap by an additional horizontal incision. **(H)** Further removal of bone with a hatchet (workplace safety). **(I)** Caudal aspect of the brain surface after removal of the skullcap. **(J, K)** Removed brain (dorsal **(J)** and ventral **(K)** aspect). Ruler length = 10 cm in sections of 1 cm. **(L)** Caudal aspect of the head after removal of the brain. Note the large convexities for the prominent temporal lobes of the brain. **(M)** Dorsal aspect of the middle cranial fossa. *Asterisks* indicate optical nerves. The *arrow* marks the position of the (left) trigeminal ganglion. The *dotted line* indicates the incision line for extraction of the pituitary gland, which is covered by a thick layer of dura mater. **(N)** Excised pituitary gland. **(O)** Excised trigeminal ganglion. Ruler length = 10 cm in sections of 1 cm. **(P-R)** Frequently observed pathological findings include purulent sinusitis of the frontal sinus (**P**) and the maxillary sinus **(Q)**; **(R)**: detail enlargement of the *boxed area* in **(Q)**.

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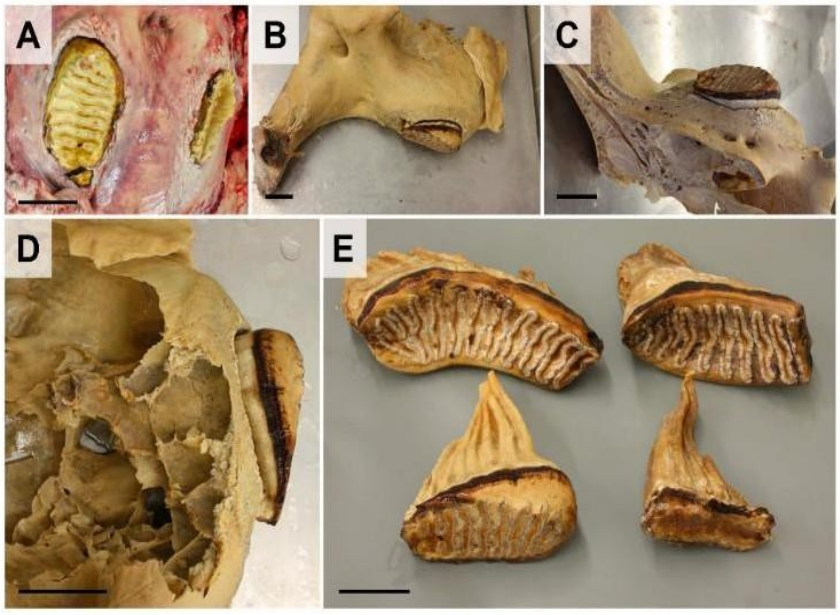

Fig 14. Teeth dissection. (A) Teeth are macroscopically inspected for lesions (tooth decay, fractures, abrasion, *etc.*). **(B-E)** Extraction of teeth is facilitated by decocting of the jawbone sections. **(B-D)** Decocted fragment of the upper jaw. Lateral **(B)** and medial **(C)** aspect. **(D)** Teeth are accessed from the alveolus and removed. **(E)** Extracted molar teeth of a 64-year-old female elephant. Upper row: Left and right molar teeth of the upper jaw. Lower row: Left and right (severely abraded) mandibular molar teeth. Bars = 10 cm.

https://doi.org/10.1371/journal.pone.0338783.g014

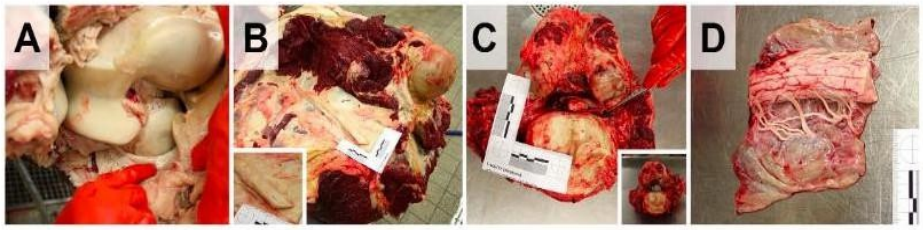

Fig 15. Dissection of large joints of the limbs (A, here: knee joint), skeletal muscles and peripheral nerves (B, here: sciatic nerve), and spinal cord segments (C, D). (C, D) Caudal aspect of the excised fourth cervical vertebra. The spinal cord is removed from the vertebral canal. See text for details. Ruler length = 10 cm in sections of 1 cm.

https://doi.org/10.1371/journal.pone.0338783.g015

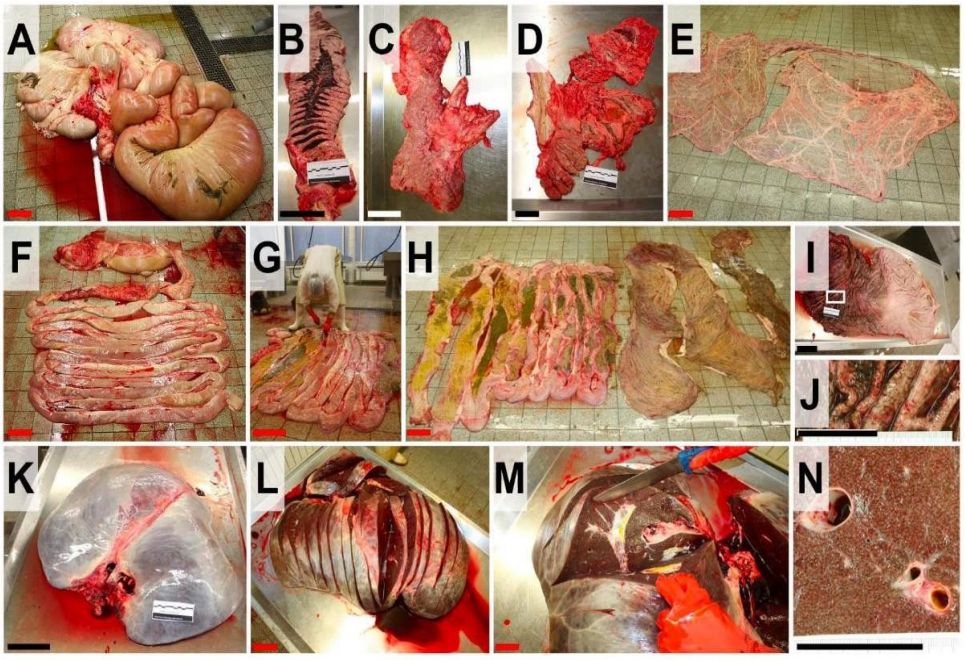

Fig 17. Dissection of the spleen and the gastro-intestinal tract. (A) Intestinal convolute after removal from the abdominal cavity. Bar = 15 cm. **(B)** Spleen, serially sliced for examination of parenchyma. **(C)** Excised pancreas with adjacent vessels. **(D)** Serially sliced pancreas. Bars in B, C and D = 10 cm. **(E)** Omentum and mesentery spread out for gross examination. Bar = 15 cm. **(F-H)** After removal of the mesentery, intestines are displayed in rows **(F)**, longitudinally opened **(G)**, and examined (**H**) after removal of the ingesta/feces. Note the huge caecum (hindgut fermentation). Bars = 15 cm. **(I)** Opened stomach (Bar = 10 cm) with hyperemic/congested gastric mucosa (**J**, Bar = 5 cm). **(K-N)** Dissection of the liver. The liver (**K**) is serially sliced (**L**) for gross examination **(M)**. Bars = 10 cm. **(N)** Closer view in the section surface of the liver parenchyma, demonstrating a distinct lobular pattern. Bar = 5 cm.

https://doi.org/10.1371/journal.pone.0338783.g017