Necropsy of Wild Animals

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The "Necropsy Procedures for Wild Animals" is also available in Portugese, French, and Spanish from the Wildlife Conservation Society website (www.wcs.org/science/wildlifehealthsci/fieldvet/fvp-techpages.html).

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I. INTRODUCTION. Why necropsy wild animals?

Disease is one of many factors affecting the viability of wild populations. In a balanced ecosystem, most populations survive with low levels of disease or with periodic epidemics. However, as wildlife populations become more dense from habitat restriction, the risks of a catastrophic epidemic within wildlife populations increase. Transmission of diseases between wild and domestic animals also becomes more likely.

To determine the disease risks to a population, the causes of morbidity and mortalities in that population must be identified. Risk assessment also includes an understanding of the natural history of infectious diseases in that environment, including the history of previous epidemics. Many wildlife disease epidemics affecting valuable wildlife resources or livestock have gone undetected because appropriate samples were not collected for diagnostic testing from animals that died during the epidemic. When appropriate samples and accurate written and photographic records are taken, the cause of an epidemic can be determined in most cases.

While it is ideal to transport sick or recently dead animals to a pathology laboratory for necropsy by trained personnel, in most circumstances transport is not possible. However, appropriate tissue samples can be obtained by field personnel if trained in necropsy procedures and sample collection. The purpose of this manual is to provide practical guidelines for performing field necropsies on wild animals and for collecting, storing and shipping samples in the field for diagnostic testing.

We strongly recommend that complete tissue and blood samples be obtained from carcasses . If only selected samples are taken because a particular disease is suspected and the animal does not have that disease, these samples may be inadequate to test for other diseases that might be causing the epidemic. Furthermore, selective sampling limits the information that could be procured from a wild animal necropsy that could aid in future population or ecosystem management.

Before performing a necropsy on an animal two important points need to be considered:

1. ZOONOTIC DISEASES : Could this species have a disease that is transmissible to humans? Diseases such as rabies or echinococcosis (Hydatid disease) in carnivores, anthrax or rabies in ungulates, or psitticosis in birds can cause serious and fatal diseases in humans. Many primate diseases also can cause human illness. FOR THIS REASON, THE PERSON PERFORMING THE NECROPSY SHOULD WEAR A MASK AND PROTECTIVE CLOTHING. Wearing a mask is particularly important when preforming a necropsy on a primate, bird, or a carnivore suspected of rabies or hydatid disease. Also, all samples should be handled with care and unfixed samples should be placed in LEAKPROOF containers so that dangerous infectious materials do not leak during transport.

2. REPORTABLE AND INFECTIOUS DISEASES : Could this animal have a disease that is infectious to livestock or other wild animals? Diseases such as anthrax, foot and mouth disease, or tuberculosis can spread to other animals through contamination of the environment during the necropsy procedure. Anyone necropsying wild animals should be aware of the typical lesions of these diseases and take extra precautions when decontaminating a necropsy site. Suspicious carcases should be deeply buried to prevent scavenger access if anthrax is suspected.

IMPORTANT DISEASES TO RECOGNIZE

The following important diseases have lesions that can be recognized at necropsy and should be handled appropriately. Note that animals with rabies do not have any typical gross lesions.

ANTHRAX

Ruminants have a large, dark “tarry” spleen; Carnivores have a swollen head or neck due to edema in the soft tissues. Blood smears contain large bacterial rods (3-10 µm long by 1 µm wide) with blunt ends surrounded by a capsule. Bacteria occur singly or in short chains. Carcasses with anthrax should not be necropsied.

ECHINOCOCCUS (“Hydatid disease”)

Clear cysts in the liver of carnivores or rodents

TUBERCULOSIS
Nodules in the lungs, enlarged lymph nodes or thickened intestinal wall.

Equipment

A basic necropsy kit can be assembled in preparation for transport to a field necropsy site on short notice. The kit should contain the following items:

Protective clothing :
Rubber Gloves
Rubber Boots or Plastic Foot Protectors
Rubber Apron
Coveralls
Mask (to cover mouth and nose) and Eye Goggles or Face Shield

Necropsy documentation
Camera and film
Field notebook

Necropsy equipment
Sharp knife (including sharpening stone or steel)
Scissors (small and large)
Forceps
String
Ax or hatchet
Hack saw or bone saw
Small and large shears
Chisel and mallet
"Come-along" or block and tackle
Scalpels and razor blades
Alcohol lamp or gas burner for sterilizing instruments
Plastic ruler or measuring tape

Specimen containers and sampling equipment
Rigid plastic containers with tight fitting lids (approximately 1 liter)
Small vials, tissue cassettes, or tags to identify specific samples
Sterile vials or blood tubes
Plastic bags with closure tops (zip-lock or whirl-pack)
Parafilm or sealing tape
Aluminum foil
Sterile syringes and needles (20 g)
Sterile swabs in transport tubes
Labeling tape or tags, waterproof labeling pens, and pencil
Microscope slides and slide boxes for transport
WHO rabies kit (or drinking straw in a small jar of glycerin)

Transport materials
Ice coolers
Leak-proof, break-proof containers
Absorptive packing materials
Sealing tape
Sterile buffered glycerin (50%) (see Appendix I for formulation)

“Easy blood” (see Appendix I for formulation)

Fixatives
10% buffered formalin (see Appendix I for formulation)
100% acetone for cytologies
70% ethyl alcohol for parasites

Disinfecting materials
Pail and brush
Disinfectant
Borax
Sodium hypoclorite (0.5%) (10% Chlorox)
70% ethyl alcohol (for disinfecting instruments)

Equipment
Microscope: A microscope with a mirror for a light source or adapted for car batteries (A field scope will permit assessment for anthrax before opening a carcass).
Centrifuge: A portable centrifuge for spinning blood is optimal. (Eg. Mobilespin centrifuge from Vulcon Technologies, 718 Main, Grandview, MO 640330 USA; 816-966-1212 or FAX 816-966-8879)

Safety Precautions

Personal safety
Because some diseases of wildlife can cause serious illness or death in humans, all carcasses should be handled as if they were harboring potentially dangerous diseases and precautions for personal safety should be exercised. Minimal protective clothing includes coveralls, gloves and a mask that covers the nose and mouth, rubber boots. When necropsying a primate, a full face shield, coveralls, and double gloves should be worn. A washable rubber apron also is recommended.

Carcass handling and disposal
Diseased wildlife also should be handled to minimize exposure of other wild and domestic animals. If ANTHRAX is suspected, a blood smear should made by nicking an ear vein or other available vein and checking for Bacillus anthracis by microscopy before the carcass is opened. Carcasses with anthrax or other infectious diseases should be buried (preferably covered with a disinfectant and buried at least 2 m deep to prevent scavenging).

Shipping samples
Fresh and frozen samples should be packaged so that no leakage occurs.

Preparing Sample Containers

All containers, tubes, slides, and bags should be labeled using a waterproof marker. Placing a second label in a plastic bag that is then attached to the container adds further security. For formalin-fixed tissues, a paper label with the animal identification written in pencil can be submerged in formalin with the tissues.

The following information should be included on the labels:
Date
Geographic location (Park name or nearest town, country)
Species
Sex and approximate age
Tissue Identification (this is not necessary for formalin fixed tissue samples)
Person taking sample
Animal ID (if available)

Performing for the Necropsy: General Considerations

Checking for Anthrax

Determine if ANTHRAX is present

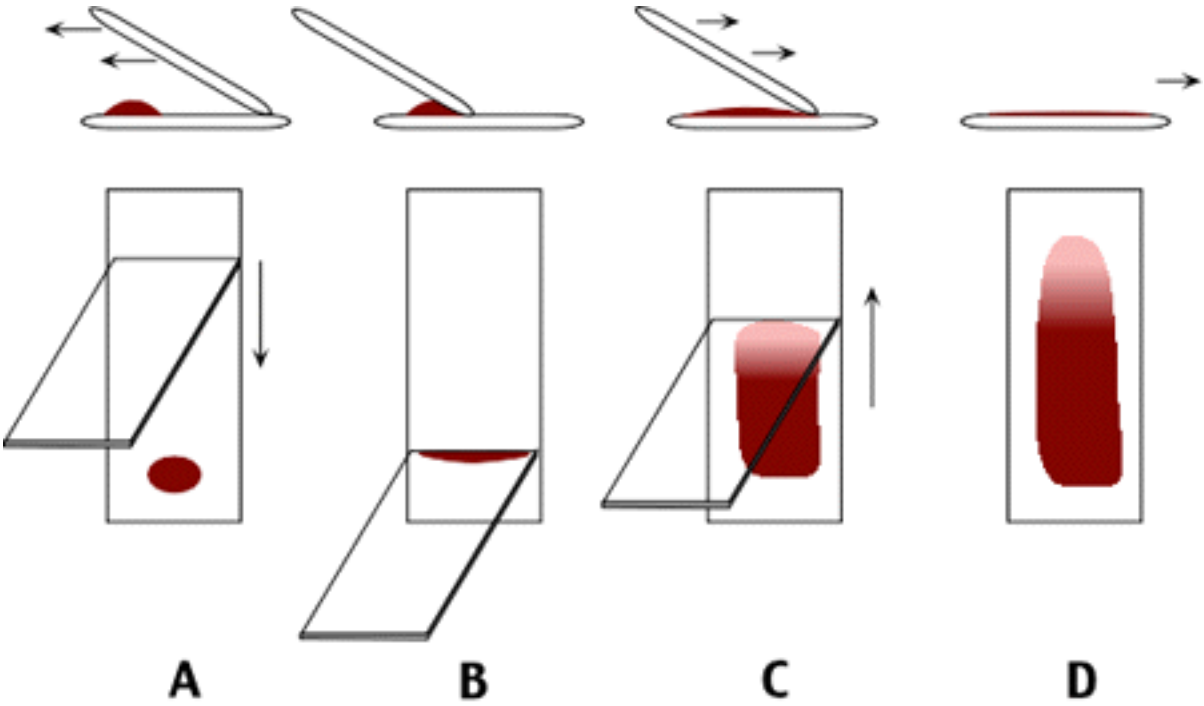
Blood Smear Procedure

Before opening a carcass, blood smears should be obtained from peripheral areas (in ungulates) or from areas of swelling (in carnivores).

IF ANTHRAX BACTERIA ARE SEEN ON BLOOD SMEARS, THE CARCASS SHOULD NOT BE OPENED.

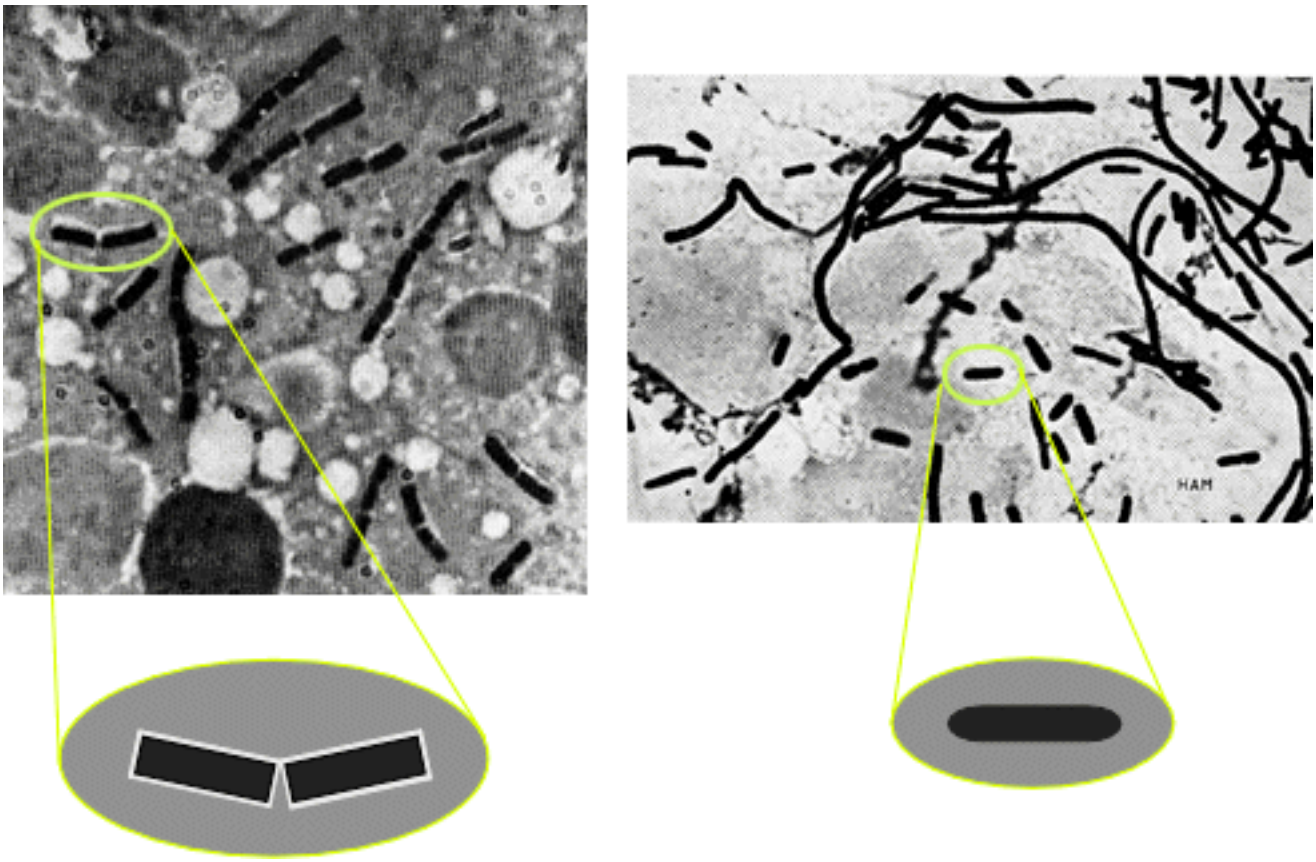
Opening a carcass with anthrax will cause the bacteria to sporulate and disperse the spores throughout the environment.

To screen for anthrax before a carcass is opened, a small amount of blood should be obtained from an ear vein or coronary band. In carnivores, blood should be obtained from areas on the face and neck that are swollen.



Take a single small drop of whole blood and place it near one end of the microscope slide (A). Bring the end of a second slide (held at a 45 degree angle) up to the drop until the drop disperses along the edge of the second slide(B). Then with the first slide placed on a flat surface, push the second slide quickly and evenly toward the opposite end of the first slide (C). The results should appear with an irregular thin edge, as illustrated (D). Several thick blood smears should also be made by spreading a drop of blood in a small circle on the slides. When the smears are dry, label one end of the slide (with a solvent resistant pen or pencil) with the date, species, animal ID, and location. Slides can then be transported to a laboratory or stained in the field with New Methylene Blue or a Wright's stain kit.

Anthrax Bacteria Diagram



A. Bacillus anthracis, X2000.
Note 2 main characteristics:
1. sharp, squared ends
2. pale capsule around bacteria
B. Clostridium septicum, X2250.
Note 3 characteristics:
1. smaller size
2. no capsule
3. rounded ends

Anthrax bacteria are commonly confused with Clostridium bacteria (see fig. above). Anthrax bacteria appear as large (up to 10 µm long) rectangular rods, alone or in chains and surrounded by a capsule. Anthrax bacilli obtained from a recently dead carcass usually do not form spores.

Handling Decomposed Carcasses

Examining the Carcass and its Environment

Assess the Condition of the Environment

Note recent weather conditions that could have caused animal deaths (drought, floods, electrical storm, etc)
Note the ambient temperature
Note signs of struggle

Assess the Condition of the Animal

Note any bite WOUNDS or other signs of predation. If wounds are present, look for bruising and bleeding in the tissues near the wounds which would indicate that they occurred before the animal died. Otherwise these wound most likely were caused from the carcass being scavanged.
Look for broken bones, missing hair, broken or missing teeth or other signs of trauma.
Look for and preserve any external parasites.

Determine Nutritional Status of the Animal

Take weight (if possible) and/or body length and girth. Assess fat stores under the skin and in body cavities.
Note the amount of fat around the heart and kidneys
Note the muscle mass of the animal
Note the amount of food in the digestive tract
Note the condition of the teeth

Most carcasses will have some AUTOLYSIS, but diagnostic tests can still be performed if tissues are properly handled.

Handle autolyzed tissues for histopathology very gently
Hold tissues at the edges only
Cut with a sharp knife or scapel
Quickly place in formalin
Freeze or refrigerate samples as soon as possible for infectious disease or toxicology testing.

Autolysis can cause many artifacts in tissues that can be confused with a disease process. However, it is always best to take a sample from an area that looks abnormal rather than assume that the change was caused by autolysis. Histopathology will be able to distinguish between true lesions and post-mortem changes.

Tissue Sampling Procedures

Histology

(see Tissue Check List in Appendix II)
Samples should be taken from all major organs and ANY ABNORMAL AREAS
All samples should be placed in a common container of 10% buffered formalin
SUBMERGE TISSUES IN 10 TIMES THE VOLUME OF FORMALIN AS THE VOLUME OF TISSUE
Samples should be no thicker than 1 cm so that they can fix, but long and wide enough to represent the different areas of a tissue as well as any abnormalities. Samples that include abnormal areas and surrounding normal areas are best.
Samples should be handled carefully by grasping at the edges. Do not scrape surfaces of tissues or compress them with forceps.
Most tissues do not need individual labeling. If a tissue needs special labeling (eg. a specific lymph node), place it in a different container (or tissue cassette) or attach a piece of paper to the tissue with string or a pin and label the paper or container with pencil or waterproof marking pen.

Microbiology (Bacteriology and Virology)

To take samples without contaminating them, the samples need to be taken BEFORE tissues are touched and the instruments need to be sterilized. These samples also should be placed in sterile containers. To sterilize instruments: dip the tips in alcohol and then flame them or flame the tips until they are red and then let them cool. Samples also can be taken with a sterile swab, sterile syringe, or by placing a large (3 cm2) section of tissue in a sterile container (the center of the tissue will be uncontaminated).

Take samples that contain abnormal areas . Appropriate samples include: whole blood, pus, areas with abscesses or nodules, or intestinal contents (within a loop of intestines).When taking samples from infected tissues, select an area near the edge of the affected tissue where live organisms are most likely to be found. If no abnormal areas are present, take standard tissue samples of lung, liver, kidney, spleen, tonsil, and intestines (see Check List in Appendix II)

Keep samples moist with sterile transport media, sealed in a sterile container and cold. If refrigeration is not available, samples can be placed buffered glycerin.

Smears of pus or infected tissues also are useful and can be air-dried and shipped with cultures.

Serology

Serum should be placed in sterile tubes then stored and shipped frozen.

OBTAINING SERUM FROM A CARCASS

In recently dead animals, the right heart usually contains plasma clots (yellow/tan material), unclotted blood, or clotted blood. Plasma or blood should be removed and left undisturbed for approximately 30 min to encourage clot formation, then centrifuged at approximately 2000 X G for 20 min. The Mobilespin centrifuge from Vulcon Technologies, 718 Main, Grandview, MO 640330 USA; 816-966-1212 or FAX 816-966-8879 is portable and easily adapted for field use. When a centrifuge is not available, serum can still be obtained by letting the clot or blood cells settle. The serum (clear/yellow fluid or red-tinged fluid if the animal is autolyzed) or plasma should be separated for the blood cells, divided into at least two aliquots, transferred to vials, and then refrigerated or frozen (-20° or -70°) until transported to a laboratory. Serum vials should be labeled with the species, animal ID, date, and owner (e.g., country and park) using a waterproof marker.

If a centrifuge is not available and blood is obtained from a live animal or a dead animal whose blood has not yet clotted, remove whole blood into a blood tube, let the blood clot with the tube inverted (rubber stopper down), then turn the tube right side up and very carefully remove the stopper with the blot clot attached, leaving the serum in the tube.

Toxicology

Take samples and place half of each sample in aluminum foil and half in plastic bags or containers (aluminum or plastic interfere with the testing of some toxins). Samples should be stored frozen (if possible) until shipped to a laboratory.
(see check list in Appendix II)

Parasitology

Make at least 3 blood smears on clean glass slides (see procedure under " Checking for Anthrax ."
Fix approximately 2 g feces in 70% ethyl alcohol or formalin.

Making Slides for Cytology

Make a clean cut with a scalpel blade across the surface of the abnormal area of the tissue you wish to examine.
Grasp the sample firmly with forceps, placing the cut surface down.
Blot the cut surface of the sample across a paper towel or other absorbent surface until no blood or fluids are evident.
Then gently touch the blotted surface in several locations on clean slides.
Air dry slides.

General Steps to Performing the Necropsy

Carcass Dissection Using the Carnivore as an Example

Introduction

The necropsy method outlined below provides a simple consistent method to examine a carcass and its body organs. A CARNIVORE has been used to demonstrate carcass dissection and tissue sampling procedures. Procedures for necropsy of UNGULATES, BIRDS, and REPTILES also are demonstrated. Very small animals (less than 100 g) can be fixed whole by opening the body cavity and submerging the entire animal in formalin.

All carnivores and ungulates are placed ON THE LEFT SIDE so that the right side of the carcass is opened. All birds, reptiles, and primates are placed ON THEIR BACK.

After the body cavities are opened, the general nutritional condition of the animal and location of all organs should be assessed (to determine if any organs are displaced) before organs are removed. At this time, a sterile blood sample for culture can be removed from the heart (the right atrium is the best location), then additional blood can be taken to obtain serum for serological tests. Also sterile samples of other organs should be taken for culture before organs are handled.

After the general condition of the animal has been recorded, individual organs can be removed, examined, and sampled in a systematic manner. Any abnormal findings (lesions) should be described. Photographs of abnormal findings provide the best documentation for records.

Describing Abnormalities Found at Necropsy

Any abnormality should be described by the following criteria:

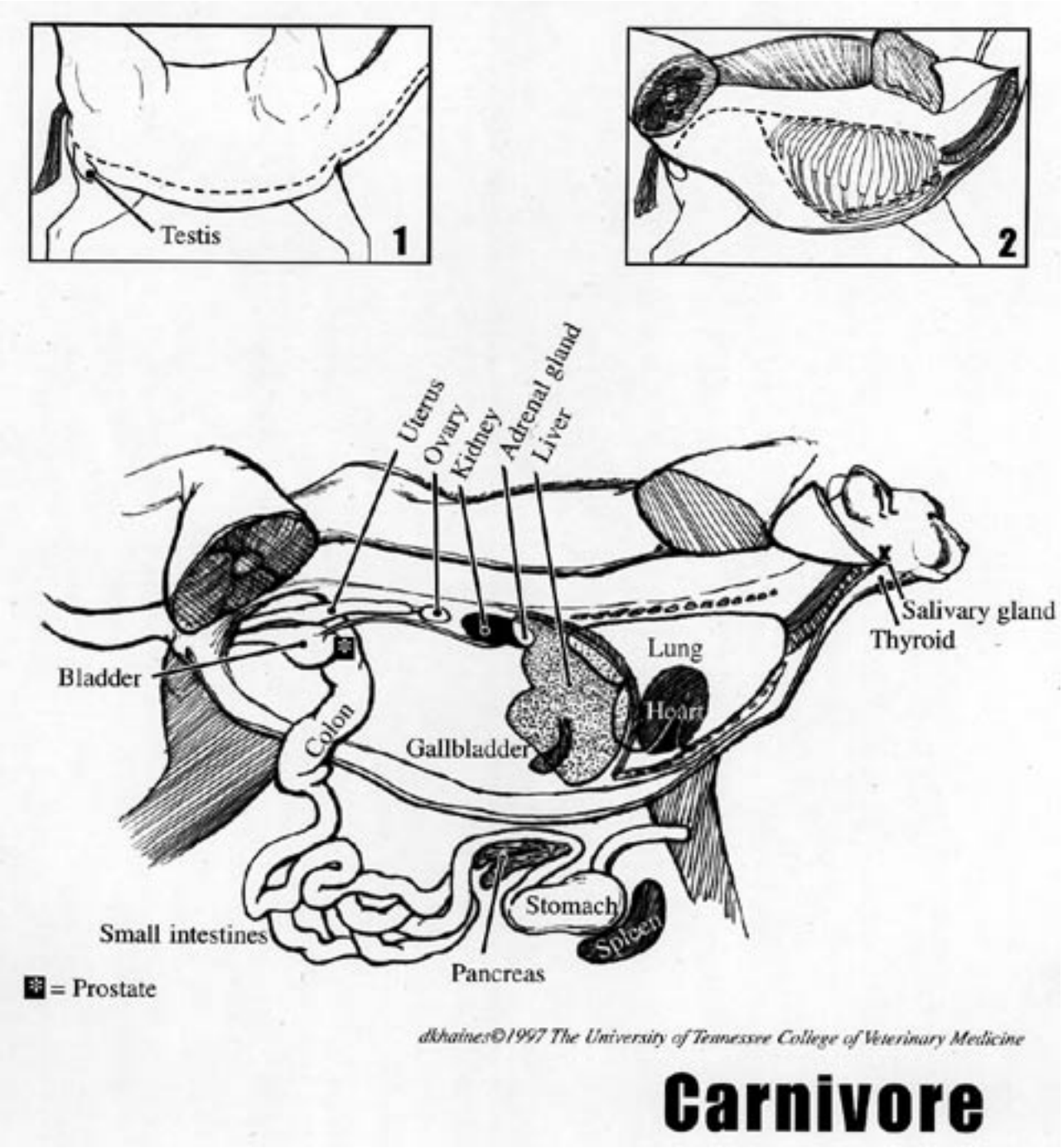
Location
Number and distribution
Color
Size
Shape
Consistency and texture

For example: “The liver contains multiple tan, firm nodules ranging from 1 to 3 cm in diameter that are distributed throughout all liver lobes. The nodules are gritty on cut surface.”

Procedure for Dissecting Carnivores (See check lists in the Appendix IIb for tissues to sample)

Figure 1. Opening the Carnivore Carcass

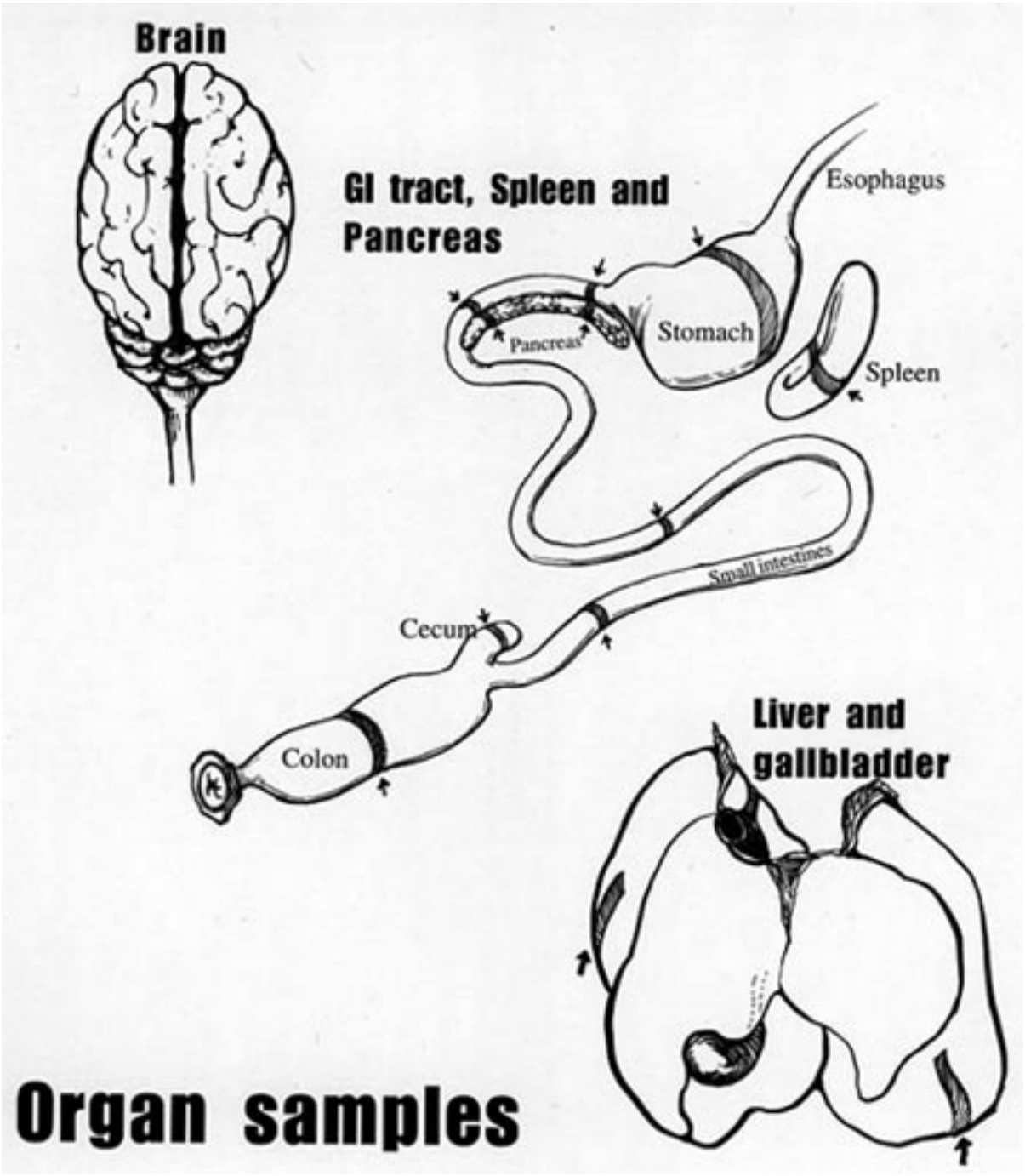
Examine the carcass for wounds and note the general condition of the fur.
Place the carcass on its left side.
Cut the skin along the ventral midline from the chin to the tail (Step 1, Fig. 1 below). On females, examine the mammary glands. On males, examine the prepuce and penis. On neonates, examine the umbilicus.
On the right side, reflect the skin to the level of the backbone.
Reflect the right limbs by cutting through the muscles and the hip and shoulder joints.
Open all three body cavities (abdomen, chest, heart):
Remove the flap of muscle and other tissues that cover the right side of the abdominal cavity.
Open the right side of the chest cavity by cutting the ribs along the sternum and backbone (Step 2, Fig.1 below).
Open the sac surrounding the heart.
Record any abnormal locations or size of organs.
Record the quantity, color, and contents of any fluids in the body cavities. Look for abnormal attachments of organs to the body cavity and determine if these attachments are easy to break.



General Organ Sampling Procedures (Figures 2-4)

Stomach and intestines (Remove stomach and intestines first but open them last to prevent contamination of the necropsy site)

Find where the esophagus enters the stomach and cut across the esophagus while holding the entry to the stomach closed to keep any food inside. Remove the stomach and intestines as a unit by cutting the mesentery where they attach to the intestines. Sample several lymph nodes along the attachments of the intestines. Leave the pancreas attached to the intestines and the spleen attached to the stomach. Cut across the rectum while holding it closed to prevent feces from escaping. Open the intestines along their length (intestines are best opened at the end of the necropsy to prevent contamination of other organs with food and fecal material). Note the content of the intestines (amount of food, presence of abnormal materials such as poisonous plants). Take stomach contents for toxicology. Take tissue samples from all areas of the GI tract and the pancreas.

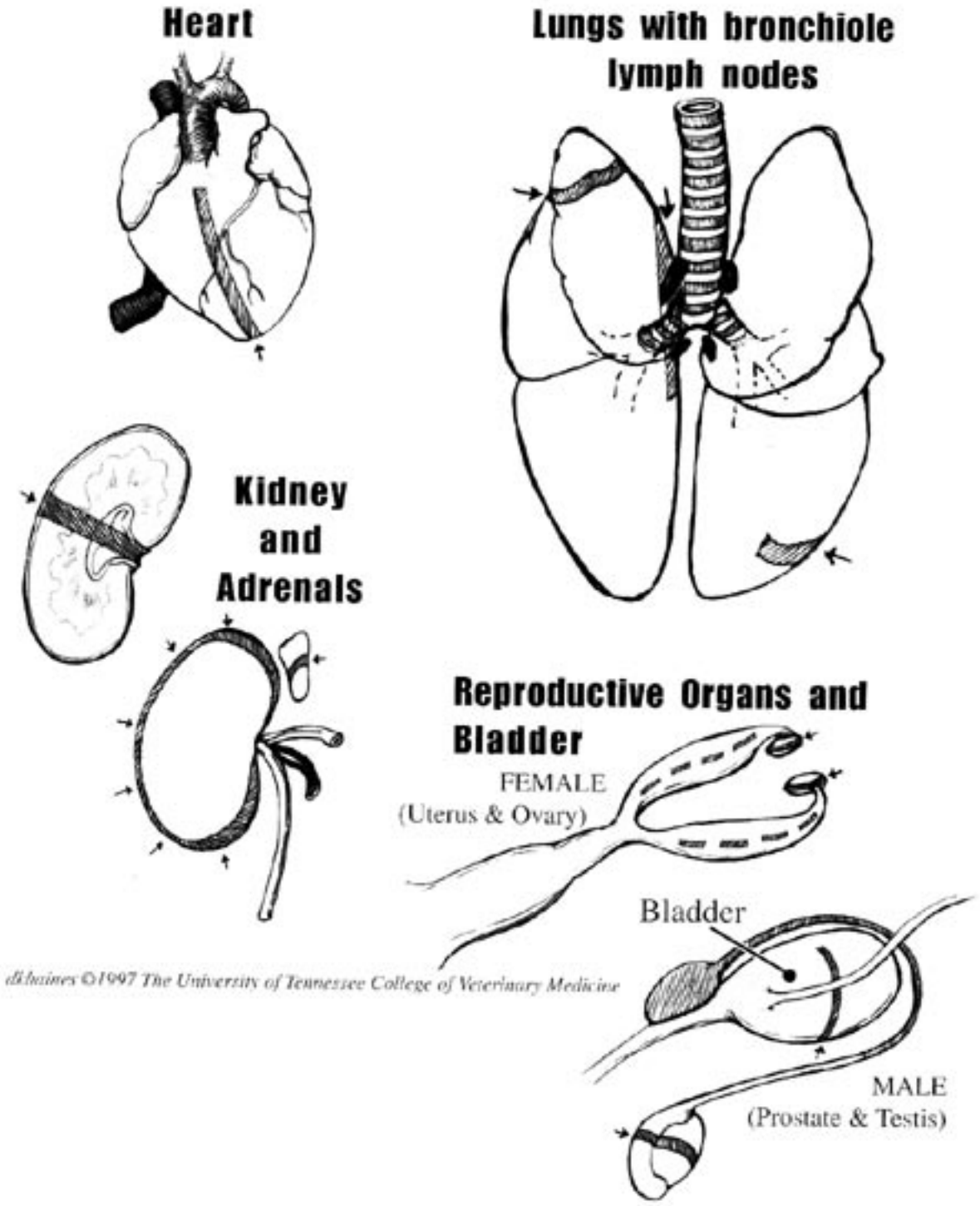


Spleen, liver, pancreas

Remove the spleen from the stomach, examine it for abnormalities by slicing it across in multiple sites and then remove samples for histology. Remove the liver; open the gall bladder. Examine the liver by cutting it across in multiple sites. Take samples for histology and toxicology.

Kidneys and adrenals

Remove and examine the adrenal glands and take transverse samples for histology. Remove the kidneys, examine them for abnormalities and take samples for histology that include the cortex, medulla, and pelvis and take samples for toxicology. Remove the bladder and take a section for histology.



Reproductive tract

Remove the reproductive tract (testis if male; uterus and ovaries if female) and make a cut across the gonads and into the uterine lumen before placing in fixative for histopathology.

Heart and lungs

Separate the bones of the larynx behind the tongue and dissect out the trachea with esophagus attached. Continue into the chest including the lungs, heart, and large blood vessels. Take a sample from the thymus if present.

Open, examine and sample the trachea and other airways and the esophagus. Take samples from lymph nodes surrounding the airways.

Examine the lungs for lumps and areas of firmness. Take samples of any abnormal areas, as well as areas that look normal.

Open the chambers of the heart and examine the heart valves between the chambers. Take samples of the heart including valve. Open the great vessels and take a sample .

Head and oral cavity

Examine the eyes, mouth and nostrils for ulcers and abnormal discharges. Remove an eye for histology by cutting the muscles around the eyeball. Cut between the lower jaw bone and tongue and remove the tongue from below. Examine the inside of the mouth, tonsils, and teeth. Remove the other tonsil and several lymph nodes under the skin at the angle of the jaw and above the larynx for histology.

Brain

To remove the entire brain: Separate the skull from the neck at the junction with the vertebra. Remove the skin from the top of the head then the top of the skull using the illustrated landmarks.

Remove the brain and cut it in half. Preserve one half in formalin and split the other half into containers for virology and toxicology.

Removing a brain if rabies is suspected
If rabies is suspected, extra precaution should be used when removing the brain. The person removing the brain should wear a face shield or mask and eye goggles. The safest procedure is to remove the head and insert a drinking straw through the hole at the base of the skull where it attached to the neck. The straw should be inserted in the

direction of the eye. Pinch the base of the straw and remove the straw with the brain sample. Then cut the straw (with the brain sample still inside) into 1 cm lengths and drop the sample in either glycerin or formalin. Although this procedure is very safe, it only allows testing for rabies. So if the samples are negative for rabies, no other brain tissues are available to test for other diseases.
The best procedure is to remove the entire brain, then cut it lengthwise down the middle. Send one half to a laboratory to test for rabies and fix one half for histology.

Skeletal Muscles and Nerves

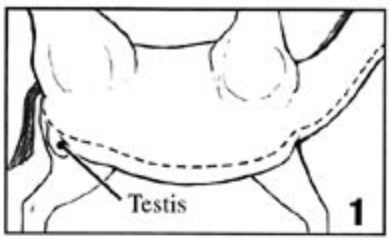
Take samples of the diaphragm and several leg muscles.
Take a sample of the large nerve between the muscle bundles of the back leg.

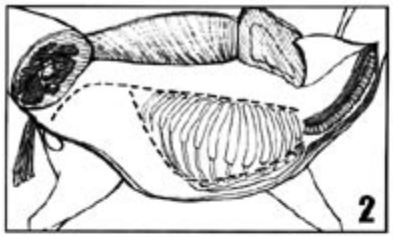
Bone Marrow

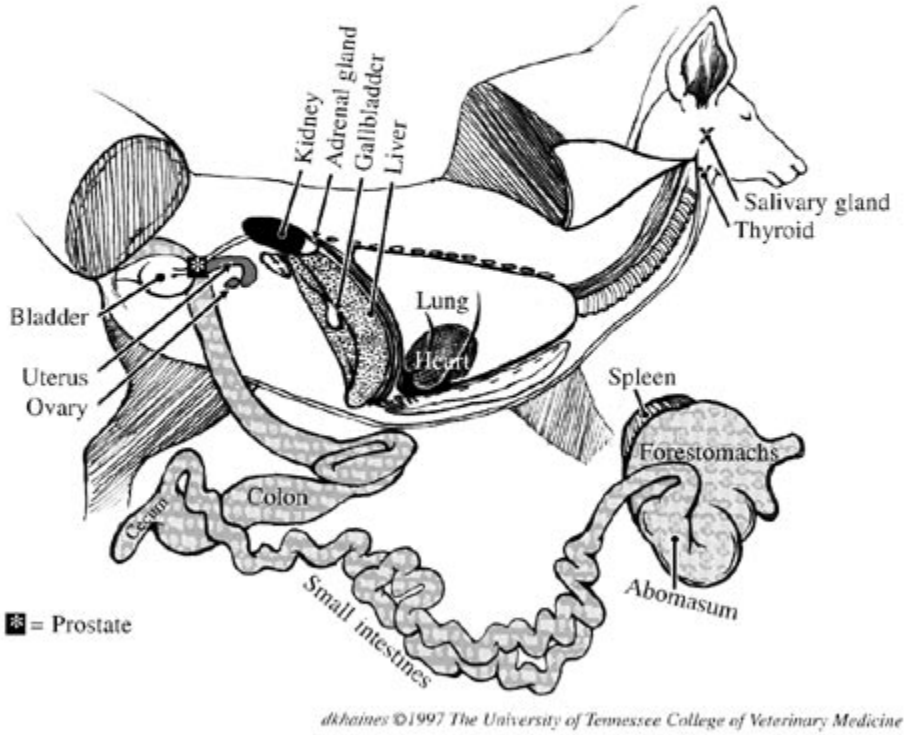
Remove a long bone from the leg and crack it open in the middle. Fix one half for histology and store half for microbiology

Ungulates

The procedures for ungulates are the same as for carnivores, except that all forestomach chambers should be opened.



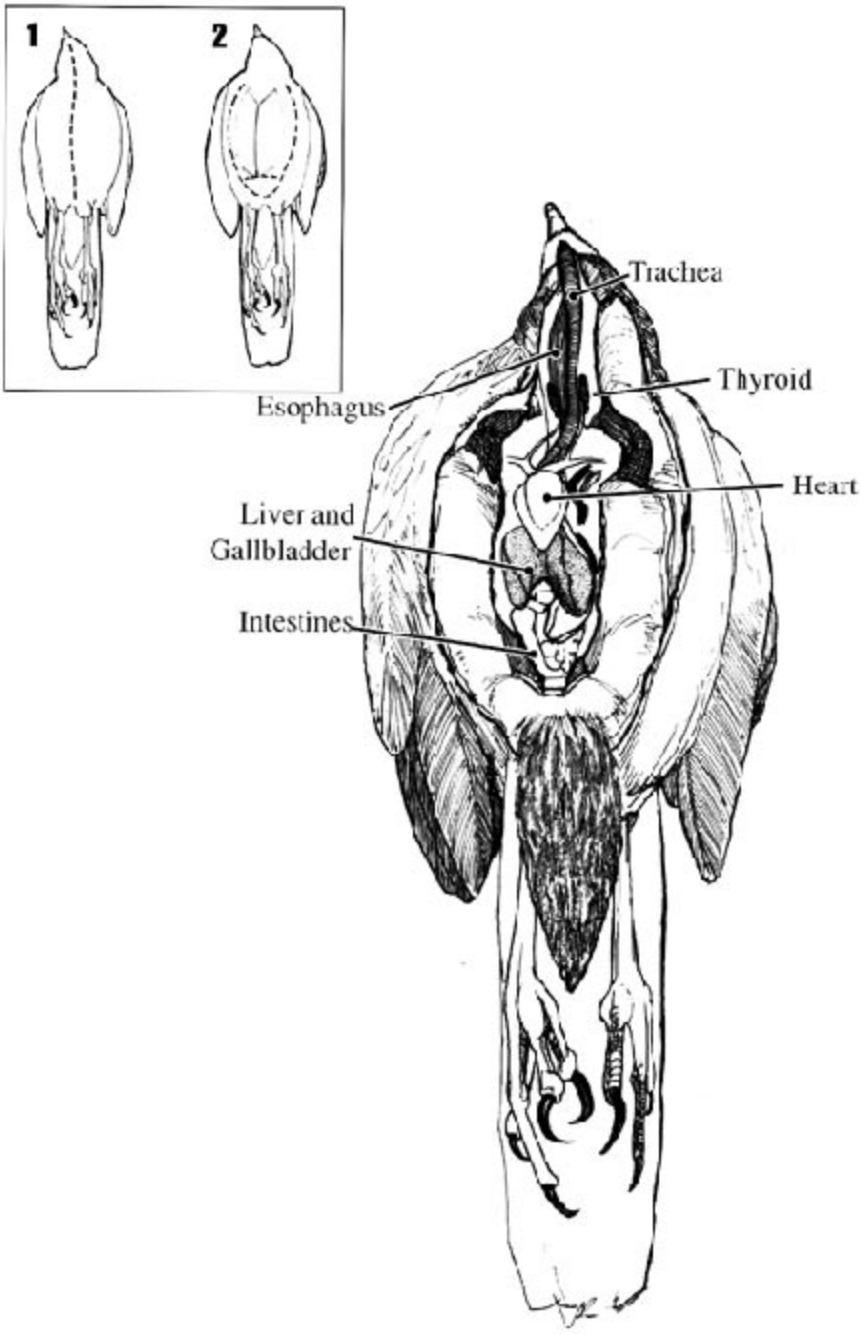




Ungulate

Birds

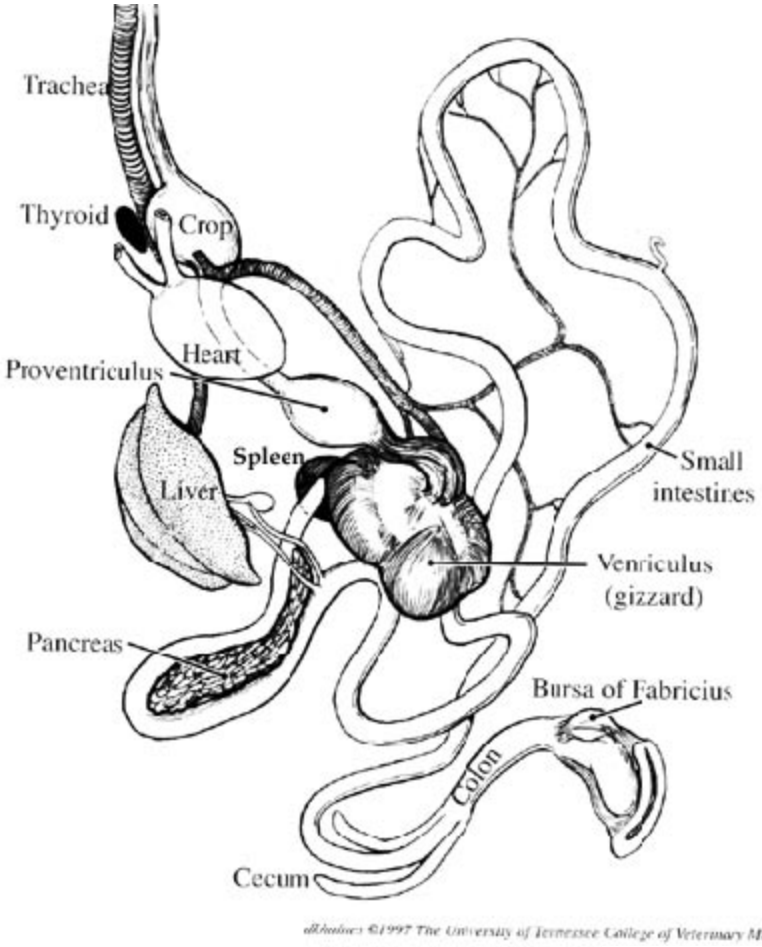
THE PROSECTOR SHOULD WEAR A MASK because humans can acquire psitticosis, tuberculosis, and fungal diseases from birds.
Dip the carcass in water containing a disinfectant or spray it to wet the feathers.
Examine the carcass for evidence of trauma and ectoparasites.
Place the bird on its back and open the skin from the beak to the vent.
Retract the skin to expose the keel and breast muscles, ribs, and muscles over the lower celomic cavity. Assess the amount of body fat under the skin and in the body cavity. Assess the amount of musculature over the keel.
Open the celomic cavity by making a horizontal cut at the bottom edge of the keel extending on each side through the pectoral muscles and then lifting the sternum. Cut the ribs and clavicle near the attachment to the sternum. Open the caudal part of the sternum long the midline.
Inspect the location and size of all organs. Examine the air sacs for transparency and note any plaques or opaque areas. Note any abnormal fluids
Using sterile instruments, take sterile samples of any visible lesions as well as of spleen, lung, and liver.
Remove the tongue, trachea, esophagus and heart as a unit. Take the thyroid glands for histology (thyroids are located at the thoracic inlet where the major blood vessels above the heart branch). Open the trachea and heart and take samples for histology.
Gastrointestinal tract, liver, and spleen: Remove the liver and take samples for histology and toxicology. Remove the proventriculus and ventriculus (gizzard) and intestines, including the cloaca and bursa of Fabricius. Note the spleen at the junction of the proventriculus and ventriculus. Fix what is remaining of the spleen (after taking a sample for culture) for histology. Open the intestinal tract along its length, noting the content and taking samples for toxicology. Leave the pancreas attached to intestines and take samples
Lungs: Dissect the lungs away from the body wall, examine them for firmness or lumps, and take samples for histology.
Reproductive tract, adrenal glands, and kidneys: The gonads (testes in the male or left ovary in the female) are located in front of the kidneys along the backbone. The adrenal glands are located just in front of the gonads and are also attached to the body below the spine. The female also should have an oviduct visible. Bluntly dissect the kidneys from the body wall, leaving the gonads and adrenals attached. Fix for histology.
Brain: Remove a section of skull covering the brain, remove a sterile sample (half of the brain, if possible) for microbiology and then fix the rest in the skull (in small birds) or remove the brain from skull before fixing (in larger birds).
Bone marrow: Remove a tibiotarsal bone and crack it open before fixing.
Nerves: Take samples from the large nerves between the wing or the leg and the body wall.



Avian Dissection

Gastrointestinal Tract, Liver, and Spleen

Remove the liver and take samples for histology and toxicology. Remove the proventriculus and ventriculus (gizzard) and intestines, including the cloaca and bursa of Fabricius. Note the spleen at the junction of the proventriculus and ventriculus. Fix what is remaining of the spleen (after taking a sample for culture) for histology. Open the intestinal tract along its length, noting the content and taking samples for toxicology. Leave the pancreas attached to intestines and take samples.



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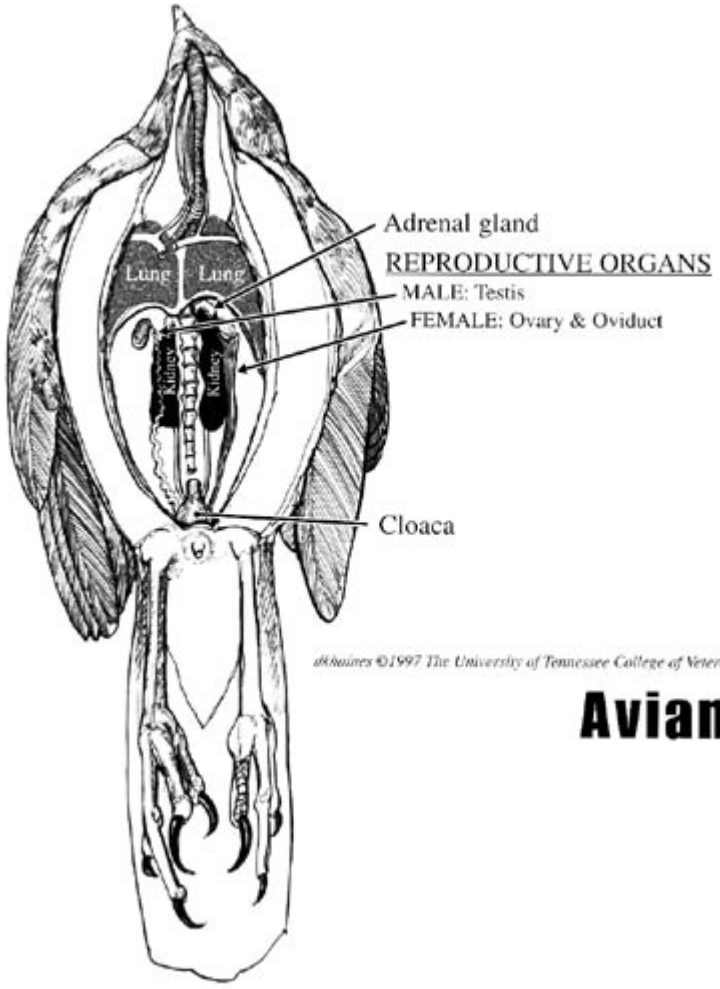
Gastrointestinal Tract

Lungs

Dissect the lungs away from the body wall, examine them for firmness or lumps, and take samples for histology.

Lungs



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Avian

Reptiles

The procedure for reptiles is similar to birds. A snake is used as an example. Other reptiles have similar organ anatomy, although the kidneys of lizards are further back in the pelvis.

The animal is placed on its back and examined for evidence of trauma. Open the body along the midline. For turtles, the plastron is removed at the junction with the carapace with a saw. The amount of body fat and condition of the musculature is assessed and any abnormal fluids recorded. Take sterile samples of lungs, liver and spleen for culture before handling the tissues.

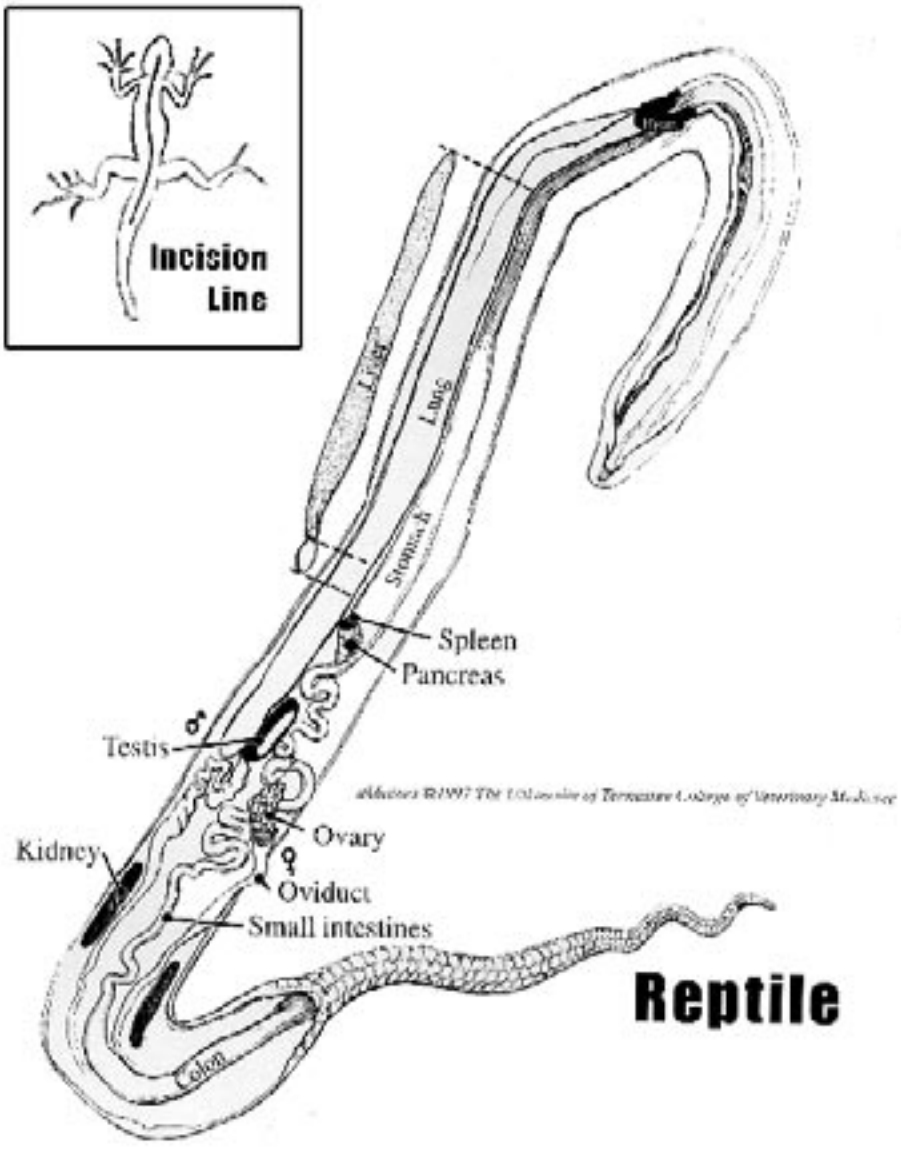
Find the thyroid(s) anterior to the heart (single midline organ in some species and paired in other species) and remove and fix them for histology.

Beginning at the mouth, remove the trachea, heart and lungs. Open the trachea and examine the lungs for firmness or lumps. Open the heart. Take samples of all organs for histology.

Remove the intestinal tract as a unit, beginning in the oral cavity. Open the esophagus, stomach and intestines along their length and take samples for histology

Remove the liver, spleen and pancreas. Examine and sample for histology

Remove the gonads and adrenals (along the midline in front of the gonads). In females, remove the oviduct with the ovaries. Dissect the kidneys from the body wall. Take samples for histology.



Reptile
Dissection Shown on Snake

Post-necropsy

Disinfecting the necropsy site

The carcass and all tissues from the carcass including blood soaked dirt should be buried or incinerated. All contaminated paper or plastic materials should be either thoroughly disinfected or incinerated. All blood and residual tissues should be removed from the instruments and tools with soap and water. Then the instruments should be disinfected. Necropsy boots and apron should be cleaned and any contaminated clothing thoroughly washed. The external surfaces of any containers with samples should also be washed.

Storage or submission of samples

TO SHIP SAMPLES:

Formalin-fixed samples can be kept at a cool room temperature until shipped.

Any samples for culture should be kept refrigerated (for parasitology or bacterial cultures) or frozen (for toxicology or virus cultures). Freezing at -70° C is preferable to 20° C (standard freezers).

1. Contact the laboratory before shipping.

2. Check regulations for shipping tissue samples. Get proper permits and use the correct containers.

3. Frozen samples must be shipped in insulated containers and by express carrier. Pack specimens in dry ice or with ice blocks. Seal container to prevent leakage. Include proper permits and animal identification.

4. Formalin-fixed tissues should be fixed for at least a week in 10 times as much formalin as tissue. Then most of the formalin can be removed and tissues can be wrapped in toweling soaked in formalin for shipping. The tissues soaked in formalin can then be placed in leakproof containers for shipping.

IT IS BEST TO SHIP FROZEN AND FIXED SAMPLES SEPARATELY. IF THEY MUST BE SHIPPED TOGETHER, then insulate the fixed tissues from freezing by wrapping in newpapers. Assure that there is no spillage of formalin, because fixation of frozen samples will make culturing for bacteria or viruses impossible and will alter cells on blood smears or cytology slides.

Dealing with an Epidemic

Complete the FIELD REPORT OF WILDLIFE DEATH form found in Appendix II.
Contact appropriate local or national governmental personnel.
Collect samples from as many dead or affected animals as possible.
Collect the following information on the epidemic:
Species and approximate number affected
Signs the animals are showing
Location of affected animals

Appendix I. Formulations for Sample Preservatives

Sterile Buffered Glycerin (50%)

For transporting tissues for culture when refrigeration is not available.

To make sterile buffered glycerin, mix glycerin with an equal amount of buffer composed of:
A. 21 g citric acid mixed in 1000 distilled water
B. 28.4 g anhydrous sodium phosphate in 1000 distilled water
Mix 9.15 ml of A and 90.85 ml of B
Mix 100 ml of buffer with 100 ml of glycerin. Then sterilize in small tubes to take into the field

"Easy Blood"

For transporting DNA from blood cells for genetic studies when refrigeration is not available. Also can be used to preserve DNA for longer periods of time if refrigerated or frozen.

1.2 g Tris HCl
3.7 g Na2 EDTA
2 g sodium dodecyl sulfate (SDS)
Add water to 100 ml

10% Buffered Formalin

For fixation of tissues for histology

To make one liter mix:
100 ml formalin (38-40% formaldehyde)
900 ml distilled water
4 g sodium chloride (table salt) [OR 4.5 gm sodium phosphate (monobasic) and 3.6 gm sodium hydroxide]

Appendix IIa. Tissue Checklist for Microbiology and Toxicology

Tissue	Microbiology	Toxicology
Brain	x	x
Fat		x
Kidney	x	x
Stomach Contents		x
Hair		x
Liver	x	x
Whole blood	x	x
Lymph Nodes	x	x
Tonsils	x	x
Spleen	x	x
Abscesses, granulomas	x	

Appendix IIb. Fixed Tissue Checklist for Histology

Preserve the following tissues in 10 % buffered formalin at a ratio of 1 part tissue to 10 parts formalin. Tissues should be no thicker than 1 cm. INCLUDE SECTIONS OF ALL LESIONS AND SAMPLES OF ALL TISSUES ON THE TISSUE LIST.

Recommended tissue sampling procedures:

__ Salivary gland
__ Oral/pharyngeal mucosa and tonsil -plus any areas with erosions, ulcerations or other lesions.
__ Tongue - cross section near tip including both mucosal surfaces.
__ Lung - sections from several lobes including a major bronchus
__ Trachea
__ Thyroid/parathyroids
__ Lymph nodes - cervical, mediastinal, bronchial, mesenteric and lumbar. Cut transversely.
__ Thymus
__ Heart - Sections from both sides including valves
__ Liver - sections from 3 different areas including gall bladder
__ Spleen - Cross sections including capsule.
__ GI Tract - 3 cm long sections of:
Esophagus
Stomach - multiple sections from all regions of the lining
Intestines - multiple sections from different areas
__ Omentum - ~3 cm square
__ Pancreas - sections from two areas
__ Adrenal - entire gland with transverse incision.
__ Kidney -cortex and medulla from each kidney
__ Urinary bladder, ureters, urethra - cross section of bladder and 2 cm sections of ureter and urethra.
__ Reproductive tract - Entire uterus and ovaries with longitudinal cuts into lumens of uterine horns.
Both testes (transversely cut) with epididymis. Entire prostate, transversely cut.
__ Eye
__ Brain - cut longitudinally along midline.
__ Spinal cord (if neurologic disease) - sections from cervical, thoracic and lumbar cord.
__ Diaphragm and Skeletal muscle - cross section of thigh muscles
__ Opened rib or longitudinally sectioned _ femur - marrow must be exposed for proper fixation
__ Skin - full thickness of abdominal skin, lip and ear pinna.
__ Neonates: umbilical stump - include surrounding tissues

Appendix IIc. Specimen Submission Form

Date

Person submitting samples:

Agency:

Address:

Phone #/Fax

Species: Animal

ID: Sex

Died Killed (circle one)

Number affected:

Location where carcass found:

Evidence of struggling?

Environmental conditions:

Other information/observations:

Appendix IId. Necropsy Protocol

Name of prosector

Address of prosector

Location of carcass

SPECIES &nb sp; ;

ANY ID # WEIGHT SEX

DATE OF DEATH DATE OF NECROPSY

HISTORY: (briefly summarize clinical signs, circumstances of death):

SHIPPING TISSUES: PLEASE OBTAIN PROPER CITES AND EXPORT PERMITS BEFORE SHIPPING TISSUES BETWEEN COUNTRIES. After 72 hrs in fixative, ship tissues in a leak-proof container in adequate formalin to keep tissues moist.

Appendix IIe. Gross Examination Worksheet

PROSECTOR:

GENERAL CONDITION: (Nutritional condition, physical condition)
Neonates: examine for malformations (cleft palate, deformed limbs etc).

SKIN: (Including pinna, feet)

MUSCULOSKELETAL SYSTEM: (Bones, joints, muscles)

BODY CAVITIES: (Fat stores, abnormal fluids)
Neonates : assess hydration (tissue moistness)

HEMOLYMPHATIC: (Spleen, lymph nodes, thymus)

RESPIRATORY SYSTEM: (Nasal cavity, larynx, trachea, lungs, regional lymph nodes)
Neonates: determine if breathing occurred (do the lungs float in formalin?)

CARDIOVASCULAR SYSTEM: (Heart, pericardium, great vessels)

DIGESTIVE SYSTEM: (Mouth, teeth, esophagus, stomach, intestines, liver, pancreas, mesenteric lymph nodes).
Neonates: is milk present in stomach?

URINARY SYSTEM: (Kidneys, ureters, urinary bladder, urethra)

REPRODUCTIVE SYSTEM: (Testis/ovary, uterus, vagina, penis, prepuce, prostate, mammary glands, placenta)

ENDOCRINE SYSTEM: (Adrenals, thyroid, parathyroids, pituitary)

NERVOUS SYSTEM: (Brain, spinal cord, peripheral nerves)

SENSORY ORGANS (Eyes, ears)

PRELIMINARY DIAGNOSES:

LABORATORY STUDIES:(List bacterial and viral cultures submitted and results, if available)

Appendix IIf. Field Report of Wildlife Death

Date:

Person reporting:

Affiliation:

Address

Telephone #/Fax

Species

Clinical signs noted before death:

Number affected:

Other species in the region/Number affected: