

Avian Necropsy Technique Transcript

Intro

This video will show you how to conduct a thorough examination of a bird carcass and how to collect vital samples that can aid in disease monitoring and detection.

Remember, every carcass can provide valuable data, regardless of its state of decomposition.

Preparation

To collect a full set of samples, you will need:

- Personal protective equipment, including a N95 respirator, eye protection, gloves, rubber boots, and protective clothing such as coveralls and/or an apron
- The proper forms to record your observations on, such as the Necropsy Form
- A camera to photograph your findings
- A ruler or measuring tape to demonstrate scale
- A waterproof marker and pencil for labeling your samples
- Sample collection containers and supplies including a container of formalin, empty cryovials, cryovials with RNAlater and/or viral transport medium, and sterile polyester swabs
- Tools such as a scalpels and handles, scissors, forceps and knives

Before you begin, make sure you have collected all relevant carcass information, including the collection location, environmental conditions, and circumstances of mortality, and that you have recorded it on the appropriate forms.

Next, organize your team. If 2 people are available, designate one person as “contaminated” and one person as “clean”. The “contaminated” team member will handle the animal, make the cuts, and take samples. The “clean” team member will NOT handle animal or any “contaminated” instruments or equipment. They will only handle clean instruments, record observations, write labels and take pictures. This structure will help with biocontainment and minimize site contamination during the necropsy.

Finally, prior to sampling, make sure to label all collection containers with appropriate identification information. All sample containers should include, at a minimum, an animal identification number, animal species, sample type, and date. Labels commonly include an ID number, animal species, sample type, and date.

Necropsy

General:

At any time during the procedure, if you see something that you think might be abnormal, or if you are unsure of whether or not it is normal, take a picture and a sample of what you think is abnormal and adjacent normal tissue. Describe your findings in the necropsy form and submit it with your other findings.

Pictures should be neat with the area of interest centered and in clear view. They should be well-lit and include a tag with the date, the animal's ID, and a section of measuring tape or a ruler for scale.

For observations, simple language with descriptions of color, size measured as length, width, thickness, and consistency are best for normal and abnormal findings. Also, remember that descriptions are not interpretations. Interpretations are based on all of the information and photographs that were gathered during the necropsy and depend on having good descriptions and photographs. Your partner veterinarian or pathologist can help with the interpretation of your findings.

External Exam:

Whenever possible, weigh the carcass.

Take measurements, including the wingspan and body length.

Assess and record the animal's sex, postmortem condition, body condition, and age class in the Necropsy form.

Examine the carcass from head to toe. Note any wounds, fractures, parasites, or other external abnormalities. If you find any ectoparasites, place them in a cryovial with 70% alcohol. Note if areas of trauma are or are not associated with hemorrhage. Some hemorrhage can occur post-mortem.

Take photographs of the carcass and any abnormalities you may have noted.

Next, collect oropharyngeal and cloacal swabs and place them VTM and/or RNA later.

Now you are ready to open the body. Position the bird on its back and make an incision down the center of the body, over the keel. The incision should stretch from the beak to the vent.

Avoid cutting into the body cavity.

Pull the skin back on either side of the incision to give yourself a clear view of the bird's underlying muscle and fat.

Collect a small piece of skin and muscle and place it in formalin. If genetic testing is of interest, place another sample in 70% ethanol.

Open the hip joints and expose the leg muscles and sciatic nerves. Collect a nerve and place it in formalin. Cut through the knee joint and expose the tibiotarsus, as this bone is the primary site for avian bone marrow collection. Crack off the proximal portion of the tibiotarsal bone using rongeurs and place it in formalin.

CHECKPOINT 1

Let's review. At this point you should have collected:

- Pictures of the carcass and any abnormalities that you found

- Notes, including the length and weight of the bird

- Cloacal and oropharyngeal swabs

- A skin sample

- A muscle sample
- A sciatic nerve sample
- And a bone marrow sample

Now open the coelomic cavity with a T-shaped incision, cutting along the bottom edge of the rib cage and along midline towards the vent. Examine the coelomic cavity. If any fluid is present, take photographs and note its color and consistency and measure or try to quantify its amount. Pull the body wall up and examine the air sacs, noting any areas that you cannot see through. Photograph the air sacs. Note the size of the liver. When normal, it should not extend beyond the caudal edges of the ribs.

Remove the keel by cutting the ribs and the coracoid bones on both sides and pull up on the keel, cutting any remaining connective tissue attachments, until you can remove the keel and ribs as a single plate.

Do your best to avoid touching any organs prior to sampling them.

Examine the viscera in situ and photograph the open body cavity. Note any abnormalities. Note the amount of fat in the coelom.

Now is the best time to collect sterile samples for molecular diagnostics, sterile tissue samples in VTM or RNA later, or swabs for bacterial or mycotic culture.

CHECKPOINT 2

Let's review. You should now have:

A photograph of the body cavity in situ

And sterile tissue samples for molecular diagnostics or culture.

Next, find the heart. It should sit just above the liver. From the heart, follow the carotid arteries cranially and you should find each of the thyroid glands, one on the right and one on the left. Note any abnormalities. Place the thyroid glands in formalin.

Expose the heart by cutting through its surrounding sac and take a picture of it. If the heart is <1.5 cm long, remove it and place the whole heart in formalin. If the heart is larger than 1.5cm long, remove the heart and cut through the right and left atria and ventricles. Collect longitudinal samples from the base to the apex of the heart from the left and right ventricular freewalls and interventricular septum that include the atria, ventricles, valves, and vessels in formalin.

Next, locate the liver. It should be a large brown organ that is visible on both sides of the open body cavity. Remove the liver and take a picture of it whole. Then make evenly spaced parallel cuts across it to examine the tissue, this is called bread-loafing, like slicing a loaf of bread. Note any abnormalities and take a picture of the bread-loafed tissue. Place a sample in an empty cryovial and 2 to 3 pieces in formalin. If a toxin is suspected, collect the remaining liver in a plastic bag.

Next, find the spleen. It is a small, reddish brown organ that sits just under the gizzard. Photograph the spleen and collect samples in formalin and an empty cryovial.

Now it's time to remove the gastrointestinal tract and trachea. Make an incision on either side of the tongue and the trachea and esophagus where they attach at the base of the beak. Pull up on the tongue and trachea and esophagus and cut the connective tissue around them to allow them to be lifted away from the adjacent tissues. Continue peeling the tract towards the vent in order to remove the remaining gastrointestinal organs (proventriculus, ventriculus, and small and large intestine) while leaving the lungs, kidneys, and gonads in the carcass. Remove the entirety of the tract by cutting the soft tissues around the vent. Set the gastrointestinal tract aside.

CHECKPOINT 3

At this point, you should have:

Pictures of the heart, liver, spleen, plus pictures and notes of any abnormalities that you have found.

Samples from the thyroid glands

Samples from the heart

Samples from the liver

Samples from the spleen

And a removed gastrointestinal tract.

Examine the remaining organs in situ. The lungs should be light pink and adhered to the back of the bird's body, just under where you found the heart.

Photograph the lungs in the body then remove them by carefully pulling them free from the bird's back. Lay them on a clean surface and bread-loaf them, as you did with the liver. Note any liquid and its color and consistency that oozes out when you cut them. Photograph the lungs cut into sections and place at least one piece from each lung lobe in an empty cryovial and in formalin. Note if the lungs samples float or sink in formalin.

Next, find the kidneys, gonads, and adrenal glands. The kidneys are long, oval, red/brown structures that lie along the spine beneath where the intestines previously sat. The adrenal glands and gonads lie at the cranial end of the kidneys. A male will have two testes, left and right, and females will have a single ovary and oviduct on the left side. Note any follicular activity.

Photograph the kidneys, gonads, and adrenal glands.

Collect the gonads and place them in formalin. Collect the adrenal glands and place them in formalin. If they are very small, the gonads and adrenal glands can be collected together with a portion of the adjacent kidney.

Collect a sample of kidney into an empty cryovial and one at least one sample from the left and right kidneys into formalin. If a toxin is suspected, collect the remaining pieces of kidney in a plastic bag.

CHECKPOINT 4

At this point, you should have:

Pictures of the lungs, kidneys, gonads, and adrenal glands, plus pictures and notes of any abnormalities that you have found.

- Samples from the lungs
- Samples from the kidney
- Samples from the gonads
- And samples from the adrenal glands

Next, linearize the tongue, trachea, and gastrointestinal tract on a clean surface and take a photograph.

Open the trachea by cutting longitudinally down to the bronchi, noting any fluid or abnormalities, and take a picture.

Make a similar longitudinal cut down the esophagus.

Take a cross section of the tongue, and a cross section from the trachea and esophagus and place them in formalin.

Next, continue cutting the tract longitudinally into the crop, proventriculus, and ventriculus. Take a picture of the contents and collect these in a plastic bag if a toxin is suspected. Take a longitudinal sample from the proventriculus and ventriculus together and place them in formalin.

Now open the intestine longitudinally along its entire length.

Lay the opened intestine out flat on the clean surface and take a picture of it.

Note the appearance and amount of intestinal material and collect feces in an empty cryovial. If you find any endoparasites, place them in a cryovial with 70% alcohol.

Collect a full-thickness, transverse sample of the duodenum with pancreas, jejunum, ileum with cecum, colon, and cloaca in formalin.

CHECKPOINT 5

At this point, you should have:

Pictures of the linearized and opened trachea, gastrointestinal tract including the esophagus, crop, proventriculus, ventriculus and intestines, pictures of the stomach contents, and pictures and notes of any abnormalities that you have found.

- A sample of the tongue, trachea and esophagus
- A sample of the crop, proventriculus, and ventriculus
- Multiple samples along the intestines
- And a fecal sample

Finally, you will need to collect the brain.

To do so, remove the head by cutting through the joint between the head and neck.

When the head is free, remove the skin and muscle to expose the skull.

Carefully remove the skull cap so you don't touch the brain. Take a picture of the brain.

Collect a small sample of the brain in a cryovial.

If the head is <2cm, leave the brain in the skull and place the entire head and brain in formalin..

If the head is >2cm, carefully remove the whole brain, whenever possible, from the skull and place it in formalin.

If a toxin is suspected, place ~5-10g of the brain in a plastic bag.

Finally, remove a whole eye. Do this by incising around the orbital socket.

Remove the eye.

Take a picture.

Place the eye in formalin.

CHECKPOINT 6

You should now have:

- A picture of the eye and brain

- Photographs and notes on any abnormalities that you have found

- A sample of the eye

- Samples of the brain

You are now finished collecting samples!

Make sure all of your paperwork and samples are clearly and permanently labeled.

Clean up your work area and properly dispose of the carcass and of other necropsy materials.

Clean your equipment & containers thoroughly.

Store samples frozen, on ice or in a refrigerator and ship as soon as possible.

Remember to submit the sample checklists, pictures, and forms along with the samples.

Make sure you also submit a necropsy report and follow-up with your focal point of contact.

Thank you for your help in monitoring wildlife disease. Every carcass counts!