CHICKEN POSTMORTEM TECHNIQUES AND NECROPSY SAMPLE COLLECTION

This postmortem technique is intended to be a guide for field veterinarians to help them to appreciate the necropsy techniques and sample collection for diagnosis of chicken diseases only. Since techniques for other poultry species are not included in this document, the document should not be used than intended purpose especially for teaching.

Reasons for Conducting a Post-Mortem

The main purpose of doing any postmortem is to investigate or diagnose causes of morbidity & mortality. Just in summary postmortem is visualizing the external parts for any visible changes or lesions (with recording), followed by opening the carcass and observing to obtains internal pathological lesions that help sample collection and diagnosis.

Postmortem is recommended on recently dead (not autolyzed or decomposed) or recently euthanized chickens when there is a rise in morbidity (number of sick chickens) and a rise in mortality.

All observed lesions (external & internal) should be described with brief notes on the submission form (for necropsy reporting look at annex one) according to the basic parameters of size, color, consistency, location & distribution.

Equipment for doing postmortem

Complete postmortem kit, gloves, paper towels, bin for disposal

Equipment for submitting samples

Plastic bags (Zipclosure), swabs, transport medium (bacterial, viral), tubes, sampling bottles (metal & plastic), 10% buffered formalin, cooler with ice or cold pack, syringes, FTA cards, Marking pens.

Collect flock history

Collect information on age, breed, vaccination history, medicines used, feed & feeding management, water consumption, disease frequency, mortality pattern. Observe the flock

appearance, individual birds (feathering, body condition), fecal appearance, respiratory characteristics (discharge, breathing pattern), flock distribution and activity.

Sample collection guide.

Samples that should be collected from chicken at post mortem include

Blood (various disease/ serology)

Swabs (cleft palate, tracheal, choanal) (Mycoplasma, ILT, IB, NCD, IBD,

Intestinal contents including feces

Organ or tissue samples

Blood should be collected pre euthanasia but swabs can be part of post mortem evaluation be collected pre or post euthanasia. Precise testing methodology and number of chickens to be samples should be specified based on the purpose of sample collection. For fresh samples, the tissue or organ should be placed in a sample bag and kept cool (refrigerator or cooler) immediately after collection and until samples arrive at the lab.

Post mortem procedure

Euthanasia

It is method of killing by minimizing pain, distress and anxiety but causes rapid loss of consciousness followed by cardiac or respiratory arrest.

Manual euthanasia

A. Hold chicken secure by both legs between the hocks & the feet
B. Place your other hand at the base of the chicken's head
C. Gently pull the head towards you knee and rotate your wrist/hand so that you will dislocate the neck at the point where the head & neck meet
D. Check to verify that the technique was effective by feeling the gap in the neck

External examination

Inspect the external physical characteristics of the bird:

1. Feathers & Skin

    Feathers coverage, cleanliness, skin integrity

2. Feet, legs, and skeletal health

    Toe & foot pad, joints, keel

3. Head condition

    Eye, ears, nostril, beak, comb, wattle

4. Development & body condition

    Weight, fleshing, fat deposition, uniformity

5. Cloaca & the tail

Feathers

Extend the wings and check the primary feathers for shape and abnormalities.

Inspect the feathers for stress marks (glucocorticoids/ broken line at the feather tip).

Inspect feathers for growth gaps.

Skin: Check the skin for

Signs of external parasites (mites, lice, etc.)

Scratches or other injuries

Dehydration (dehydrated birds will have dry skin and elevated scales on the legs; older dehydrated birds will have a dry, scaly comb).

Mite

Feathered Areas: Depluming Mite -burrows into feather shaft causes birds to pull out feathers

Legs and Feet: Scaly Leg Mite - raised, uneven, crusty and/or thickening scales

Fowl Mite - Black, dirty, feathers caused by accumulation of mite droppings and dried blood.

Under Wing Fowl Tick - tiny tick that sticks to skin and causes red spots

Vent Region- Poultry Lice - tiny, yellow colored. Egg clusters are attached at the base of feather shafts.

Feet, Legs, and Skeletal Health

- Evaluate the condition of the feet (foot pads, toes and toenails).
- Inspect for any litter or manure accumulation, ulcers, and foot or toe damage. Inspect the legs for overall health condition.
- With your thumb feel for hard nodules or inflammations in the Achilles tendon.
- Check for swelling of the hock joint.
- Evaluate leg straightness. Hold the legs at the natural width for the frame size of each individual bird. Holding the legs either too far apart or too close together will make them appear crooked.

The keel bone

- The keel bone is the pronounced bone that extends from the sternum and runs axially over the midline. It is located ventral to the heart and anchors the muscles used for wing motion (pectoralis major and pectoralis minor). Palpate the keel bone and inspect it for curvatures or bends.
- The keel bone also can be visually inspected after euthanasia and breast skin removal.

Head Condition

Head

- Check the head for any swelling

Eyes & ears

- Check eyes and ears for redness, swelling and discharge.

Nostrils & the beak

- Check the beak shape. Check the beak, internally and externally including the corners for abnormalities such as lesions from mycotoxins.
- Check the nostrils for blockages and buildup of materials. Apply pressure to the top of the nostril to check for mucus.

Comb & wattle

- Observe the size and color of the wattle and comb relative to age

Cloaca and Tail

Inspect the cloaca.

- Check for a pasty vent (cloaca) and accumulation of urate deposits.
- For males, check the color (redness) for active breeding roosters

Inspect the tail.

- The tail should be wellfeathered. Any signs of tail pecking can be an indication of stress, high density, or nutritional deficiencies.

Post-mortem procedure and visual examination of internal organs

It is recommended to wet the carcass using water containing a disinfectant after euthanasia to keep feathers and down from interfering with the post-mortem examination (if Avian Influenza, Newcastle Disease, *Salmonella Enteritidis* / *Gallinarum* / *Pullorum* is suspected)

Place the bird on its back with the head away from you. Incise the skin at the thigh-body wall junction, dislocate the coxo-femoral joints (the bird will now lie flat on its back).and make a small incision through the skin between the caudal end of the breast bone and the cloaca. Pull the breast skin away from you towards the neck and

- Inspect the femoral head at the dislocated coxo-femoral joints
- Examine the subcutaneous tissue, breast musculature and thigh muscles (look for any hemorrhages in the thigh muscles (mycotoxin/injury or disease like IBD).

Leg Examination

Sciatic Nerve

- On the interior of the upper thigh, raise or cut the muscle to expose the sciatic nerve.
- Expose the nerves on both legs. There should be no swelling and the nerve on the right and left should be the same size.
- Using scissors, a portion of the nerve can be excised and collected for laboratory analysis.

Joints, Tendons

- Make an incision in the hock joint.
- Carefully cut through the joint to open it fully.
- Push a finger into the joint. There should be a sticky fluid attached to the finger when slowly withdrawn.
- Cut down between the joint and the skin on the leg to reveal the tendon.

- Inspect the area between the skin and tendon for fluid accumulation. Examine the tendons. They should be white, smooth, and shiny

Head and Neck Region

- Eyes
- Remove the eyelid.
- Check the color and size of the harderian gland. The harderian gland is important for the immune function and lubrication of the eye.
- Look for inflammation of the eyelid. Look for irregularities of the cornea (clear dome over the eye), iris (colored part) and pupil of the eye

Thymus

- Examine the back of the neck especially in young chicks as day of age subcutaneous injections are placed here and may be a source of infection and early mortality issues.
- Make an incision at the base of the neck and gently remove the skin to reveal the thymus.
- The thymus consists of several pairs of pale pink, flattened, irregularly shaped lobes located in both sides of the neck positioned parallel to jugular vein and vagus nerve. The thymus lobes can be seen on both sides of the neck near the jugular veins.
- The major causes of thymic atrophy in poultry are infection with chicken anemia virus (CAV), infectious bursal disease virus (IBDV) or Mareck's disease virus (MDV).

Trachea, Esophagus, Tongue

- Cut through the corner of the beak to open the mouth and expose the entrance to the trachea and esophagus.
- Cut down to the crop.
- Open the cut. The entry to the trachea and esophagus can now be seen. Examine the esophagus, oral cavity, tongue and under the tongue for any abnormalities (such as ulcers due to mycotoxins).
- Examine the tongue for necrosis and diphtheria by toxins or pox. Take note of any raised or discolored areas. The mucosal surfaces are normally very uniform in color and appearance.

Cleft Palate, Trachea

- The cleft palate is often used for taking swab samples.

- Examine the cleft palate for excessive mucus and red mucus.
- A longitudinal section of the trachea from the syrinx (tracheal bifurcation) to the larynx will allow careful examination of the mucosa with collection of samples by swabbing (for bacterial culture or for PCR) to evaluate for important respiratory pathogens such as AI, ND, IB, ILT, *Mycoplasma gallisepticum*, *Avibacterium paragallinarum*

Crop

- Examine the crop for size (correct based on age and weight) and cut the crop to examine the inside. Evaluate the contents of the crop (i.e., feed, litter, etc.) and look for irregularities on the internal surface of the crop (i.e., fungal growth). A fungal infection by Candida can cause whitish, thickened patches inside the crop.

Brain

- Remove the feathers and skin from the head.
- Using scissors cut the base of the skull.
- Carefully cut along both sides of the skull and the front.
- Inspect the brain for any discoloration or nodules that can indicate bacterial or fungal infection. The brain should be pale in color. Brain tissue can be collected and sent for histology, culturing and/or PCR.

Thoracic Cavity and Coelomic Region

Exposing the Thoracic Cavity and Coelomic Region

- Snip the tissue at the tip of the sternum to open the thoracic (chest) cavity and coelomic (abdominal) region.
- Cut along the edge of the breast, cutting through the muscle and the ribs. Try to avoid damaging the liver, intestines and gall bladder with your knife or scissors.
- Gently raise the breast plate to reveal the liver and heart.
- Cut through the shoulder to remove the breast plate. Move the breast plate to the side or remove it completely so that you can inspect the organs. Be careful not to cut the trachea.

Heart, Syrinx

- After the breast plate is lifted or removed, the liver, heart and trachea can be seen clearly.
- The heart should feel muscular (firm) and not soft (flaccid or floppy).
- Raise the heart to reveal the syrinx (where the trachea splits into two).
- Check the syrinx for mucus/ pus, at the point where it splits.

Liver, Spleen, Gall Bladder

Examine the liver (Be careful not rupture gallbladder/antibacterial effect)

- Liver color can vary based on the diet and age of the bird. Chicks typically have a yellow liver (due to yolk absorption) while older birds have a dark red liver as shown here.
- Look for a difference in color, spots and inflammation of the capsule around the liver (perihepatitis).
- The edges of the liver should be well-defined.
- Samples of the liver can be collected using swabs or a small amount of irregular liver tissue can be placed in a sample jar to submit to the lab.
- Elevate the corner of the liver to view the gall bladder. The gall bladder releases bile fluid into the digestive tract to support fat digestion.
- The normal color of the gall bladder should be dark green. A large gall bladder would indicate a high level of hemoglobin turnover.
- Remove the liver with gall bladder and spleen attached.
- The spleen is a small, round, soft organ similar in color to the liver. The spleen is normally about 1 inch (2.5 to 3.0 cm) long and is about the size of a large olive.
- Enlargement of the spleen may indicate a high production of lymphocytes in response to an infection.

Air Sacs, Lungs, Ribs

- Grasp the gizzard and pull it to the left to reveal the intestines, abdominal air sacs and remaining internal organs.
- Air sacs should be clear and free of exudate.
- The lungs are firmly attached to the ribs in the upper thoracic cavity under the heart.
- The lungs should be a bright pink / red color), without signs of inflammation.
- Normal lung tissue is spongy and no nodules or irregular texture should be palpated
- Remove the lungs on each side to view the ribs. Check the shape and spacing of the ribs for uniformity.
- Abnormal findings may include bead like pearls on the ends of the ribs (i.e., rickets).

Gizzard, Proventriculus

- Remove the gizzard and proventriculus. Open each organ and inspect them.
- Check the gizzard for erosions and ulcerations.

- Erosion is a lesion only present in the membrane (koilin layer/lining of the gizzard).
- Ulceration is a lesion that has eroded through the lining to the muscle layer of the gizzard.

Intestines

- The GIT is excised between the oesophagus and the proventriculus and gently pulled out. Excision of the doudenohepatic ligament may be necessary.
- The pancreas is in the center of the loop of the duodenum and is normally a pale color.
- Meckel's diverticulum (yolk sac absorption site) marks the end of the jejunum and the start of the ileum.
- Evaluate meckel's diverticulum for yolk sac retention.
- A large meckel's diverticulum is indicative of a poorly absorbed yolk sac in young birds.

Use scissors to open the length of the intestinal tract in order (duodenum to ceca) to examine the intestinal content and texture

- Evaluate the opened intestinal tract for color, content and mucus presence (Mucus protects the intestinal wall from being infected with pathogens).
- In a normal healthy bird the intestine should be pale in color.
- An abnormal finding might include the presence of hemorrhages, irregular color or thickness of the ingesting, and irregular texture of the mucosal lining of the intestine.

Ceca, Cecal Tonsils

- The ceca are located where the small and large intestines join. They are only emptied on average 1 to 2 times a day.
- The ceca droppings can be seen on the floor of the poultry house and are a softer, stickier and more watery in consistency than a ‘normal’ dropping.
- Sizes may vary among birds.

Use scissors to open the ceca to examine the contents.

- The cecal tonsils are at the joint between the ceca and large intestines.
- The cecal tonsils are lymph nodules and will respond to an infection.
- Evaluate cecal tonsils for cleanliness and size.
- Normal cecal tonsils should be clean and without red inflammation.
- Abnormal cecal tonsils may be red and/or inflamed (swollen).

Bursa of Fabricius

- After removing the intestinal tract, the bursa of fabricius can be located near the cloaca.
- The bursa will normally decrease in size as the bird ages and may be difficult to locate in older birds.
- On post-mortem inspection, the bursa will appear as a small, round, pale colored sac.
- After opening it, there are several small folds inside the bursa.
- Evaluate the bursa for irregular size (e.g. much smaller than expected for the age of the bird), swelling (e.g. presence of edema and folds may not be present if bursa is swollen), and irregular coloring (e.g.: small pin-point blood spots or hemorrhages).
- In young birds, the bursa can also be examined and removed in post-mortem birds by carefully cutting the skin on the dorsal side just above the tail.

Female Reproductive Tract

Cloaca

- Inspect the cloaca for wounds, cleanness, or urate deposits.
- For hens in production, check for prolapses. Predisposing factors include (Repeated pecking at the vent, exposed oviduct following egg laying fails to retract in hens with poor conditioning., Overconditioned hens often have insufficient elasticity and tone due to excessive fat)

Ovary, Oviduct

- Examine the ovary for size based on age. Make note of any signs of bleeding.

Follicles, Stigma in Hens

- Remove the follicles. Inspect for vascularization and degeneration of follicles.
- Check follicles over 1 cm. A good range is 6 to 8 follicles, > 1 cm each, and increasing in size.
- The stigma is the point of rupture of the follicle during ovulation. If the follicle splits at a point other than the stigma, blood vessels may rupture.

Infundibulum in Hens

- The infundibulum engulfs and pulls mature ova that have been released from a follicle into the oviduct.
- Fertilization occurs after the ovum is in the infundibulum. If the infundibulum does not engulf the ovum, the ovum may be reabsorbed.
- If not reabsorbed, the ova can accumulate in the coelomic cavity and can lead to a syndrome known as an "internal layer".
- An ovum has been released and the post-ovulatory follicle is visible. The post ovulatory follicle has a hormonal function. Normal appearance should be whitish red.

Magnum, Isthmus in Hens

- Check for bleeding, ulcers, and remains of albumen or yolk

Uterus, Vagina in Hens

- The uterus and vagina with the sperm storage glands.
- Normal appearance is a light rose color. Inspect for infections and bleeding

Ovum, Egg in Hens

- An ovum located in the isthmus just before entering the uterus where shell formation, mainly calcium carbonate, takes place. An ovum at this point should have a semi-transparent membrane.
- Inspect the ovum for albumen structure.
- Normal appearance of the uterus is light pink. Inspect for bleeding and the egg for shell deformities.

Examination for Internal Parasites

- Incise the duodenum close to starting close to the gizzard and Note any abnormality, severe inflammatory (enteritis,) and examine for parasites as follows :-
- *Large roundworms* (Ascaris).-These are obvious to the naked eye, the males measuring 50-76 mm. and females 72-116 mm. in length.

To examine for small parasites(Capillaria spp, Davainea spp) scrape the intestine in two widely separated areas with a scalpel, transfer a little of the scrapings to a slide, mix with a drop of water, cover with coverslip, and examine systematically under the microscope

Caecal worms may be seen in the caeca.

The whole intestine (including caecae) should be examined for any evidence of coccidia.

- Upper small intestine (*Eimeria acervulina*): duodenum has transverse white bands to coalescing white plaques on duodenal mucosa. Moderately pathogenic occurring more commonly in older birds and often together with other *Eimerias*.
- Mid small intestine (*Eimeria necatrix*): mid intestine distended with yellow / orange mucus. White spots (schizonts) and pinpoint hemorrhages. This is a severe pathogen causing high mortality.
- Mid small intestine (*Eimeria maxima*): mid intestine and macroscopic lesions similar to *E.necatrix*. Very large oocysts are the distinguishing feature. Moderately pathogenic.
- Lower small intestine and rectum (*Eimeria brunetti*): fibrinous to fibrinonecrotic enteritis of lower small intestine, rectum and proximal part of the cecum, with necrotic core formation. Severe pathogen inducing high mortality in young birds.
- Cecum (*Eimeria tenella*): cecae filled with necrotic caseous cores. Highly pathogenic in young birds.

Citations used

1. Saif Y.M. 2008. Diseases of Poultry. 12th Edition. Blackwell Publishing, Iowa..

2. Chicken necropsy guide, The Department of Primary Industries and Regional Development, Western Australian agricultural and food industries, fisheries and regional development within thestate**, https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwiY1oWo396AAxW4cPEDHbE7CaIQFnoECBwQAQ&url=https%3A%2F%2Fwww.agric.wa.gov.au%2Fsites%2Fgateway%2Ffiles%2FA%2520visual%2520guide%2520to%2520a%2520chicken%2520necropsy.pdf&usg=AOvVaw1XVP0RSgVPx9DzQeB3bgss&opi=89978449**

Annex one

Post mortem finding collection & submission format

External examination result/finding		
N0	**Organ examined**	**Abnormal finding**
1	Feathers	
2	Skin	
3	Legs	
4	Feet	
5	Keel	
6	Head	
7	Eyes	
8	Ears	
9	Nostrils	
10	Beak / Mouth	
11	Comb / Wattle	
12	Bodyweight /Conditioning	
13	Cloaca	
14	Tail	
Internal organs examination result		
1	Subdermal Muscles	
2	Femoral Head	
3	Sciatic Nerve	

4	Leg Joints / Tendons	
5	Eyes	
6	Thymus	
7	Trachea / Esophagus / Syrinx	
8	Cleft Palate / Tongue	
10	Crop	
11	Brain	
12	Heart	
13	Liver/Spleen/Gall Bladder	
14	Air Sacs / Lungs / Ribs	
15	Gizzard / Proventriculus	
16	Intestines / Ceca / Cecal Tonsils	
17	Kidneys / Ureters	
18	Bursa	
19	Female Reproductive Tract	