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Post-Mortem Protocol for Poultry – Part 2

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Proventriculus/Gizzard

- The proventriculus, gizzard and intestine are now opened for examination of their internal surfaces. Closely examine the mucosal surface of the proventriculus for evidence of linear haemorrhages (Newcastle disease, avian influenza, infectious bursal disease) at the junction of the oesophagus and proventriculus plus proventriculus and gizzard (see stars, Figure 23).
- Examine the surface of the gizzard for both colour and the presence of erosions.

Figure 23. Proventriculus and gizzard

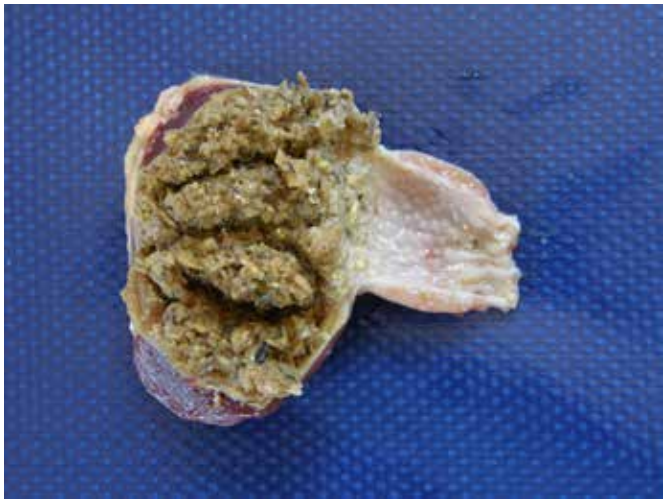

Figure 24. Normal gizzard content

- The crop and oesophagus are opened for examination of their mucosal surfaces (Figure25 and 26).

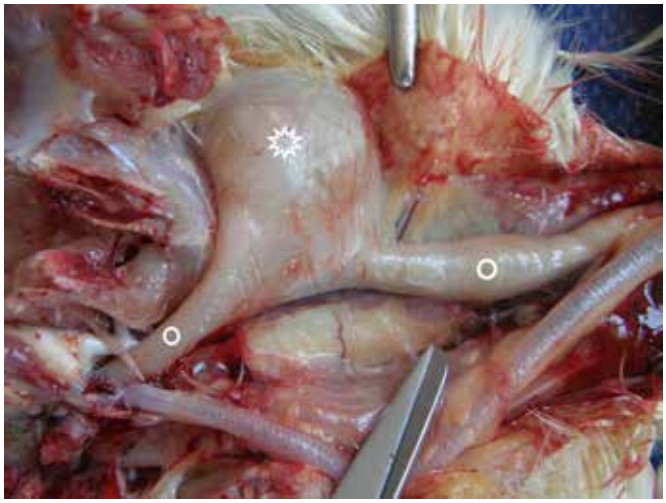

Figure 25. Crop (asterix) and oesophagus (circle).

Figure 26. Opened crop and oesophagus

Brain.

- Remove the head at the atlanto-occipital joint, peel the skin towards the beak to expose the skull (Figure 27).
- Incise the skull from the occipital foramen forward in two tangential cuts to both orbits where the incisions join (Figure 28).

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Figure 27

Figure 28

- Remove the excised cranium to expose the brain (Figure 29).
- Gently undermine the brain from the anterior region levering gently upwards and place whole brain into a formalin bottle (Figure 30).

Figure 29

Figure 30

Ischiatic Nerve

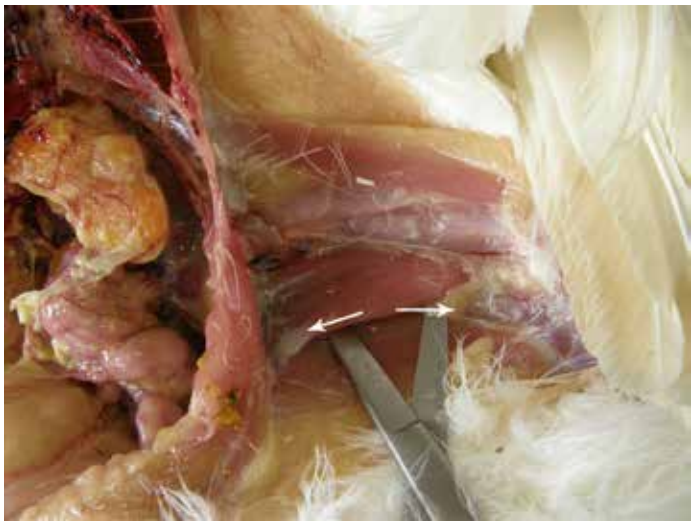

Figure 31

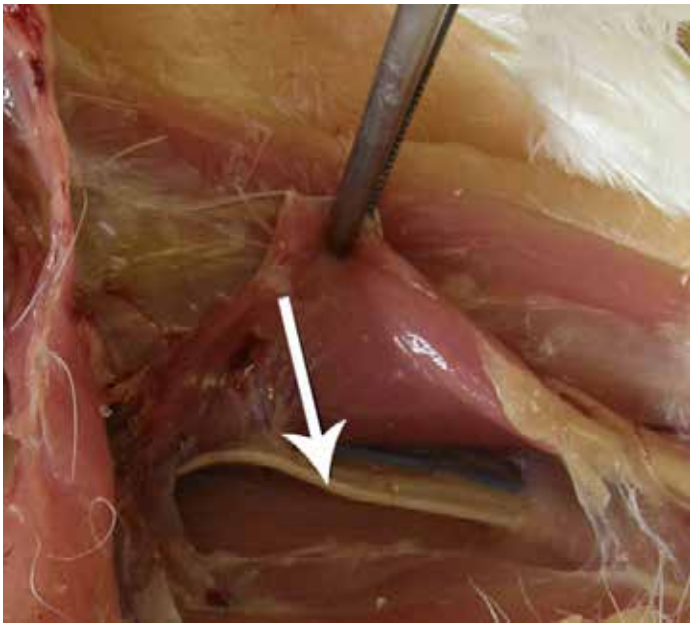

Figure 32

- This large nerve is positioned on the medial side (inside) of the femur, which is covered by a group of three thigh muscles. The nerve lies medial to the femur and below two muscles caudal to the femur.
- Closed scissors, forceps or a finger may be used to separate the two caudal muscles to expose the ischiatic nerve (Figures 31 and 32).
- Ischiatic nerve is the prime site for diagnostic lesions of Mareck's disease.

Caecal Tonsils

The paired caecal tonsils lie at the junction of the paired caeca with the ileo-rectal portion of the large intestine. They comprise a slight elongated swelling of the caecal neck (arrows, figure 33).

Caecal tonsillar haemorrhages are important indicator lesions for velogenic Newcastle disease and avian influenza.

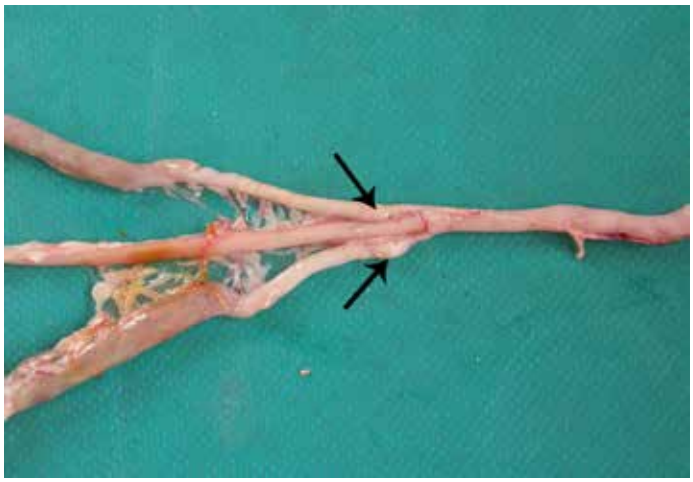

Figure 33

Leg Bones, Joints and Epiphyseal Growth Plates

Incise the hock joint from the caudal aspect and the stifle joint from the anterior aspect to examine the joint cartilage and joint fluid (Figure 34, 35).

Figure 34. Stifle joint.

Figure 35. Opened stifle joint

- In young broiler chickens, a longitudinal (sagittal) incision is made with a scalpel through the proximal end of the tarsometatarsus at the hock joint of the leg to expose the bone growth plates and bone marrow.
- Examine the proportions of the various components of the epiphyseal growth plates, namely the zones of proliferation, pre-hypertrophy and hypertrophy, for evidence of metabolic bone disease (Figure 36).
    - Calcium excess and phosphorous deficiency (widening of the zone of hypertrophy).
    - Calcium deficiency (widening of the zone of proliferation).
    - Vitamin D deficiency (hypertrophied cartilage zone of variable length)
- Examine the bone marrow for any evidence of anaemia indicative of possible chicken anaemia virus (CAV).

Figure 36

Submission Forms

- The submission form details are a critical part of any pathology specimen received at the laboratory. Remember, if the goal of your submission to the laboratory

is to get an accurate diagnosis, then you must tell the pathologist what you know and think about the case, especially what you did or did not see.
- Always include a full clinical history with relevant clinical data, e.g. bird type, flock ID, flock size, flock age, vaccination program, medications applied, feed source and type, anti-coccidial program, mortality pattern etc.
- Describe the pathology observed with short notes on the submission form. Inclusion of digital photographs of lesions noted are invaluable to the laboratory.
- Communication between the producer/poultry veterinarian and the pathologist is crucial to the diagnostic success.

Ancillary Laboratory Tests on Post-Mortem Samples

Histopathology

- Do not overfill formalin jars with tissue as this results in poor fixation, which permanently compromises histopathological examination.
- Adhering to the ratio of one part of tissue to nine parts formalin ensures good fixation of the tissue, which ensures high quality tissue sections for histological examination.
- Fixation is singularly the most important step for producing quality histopathology slides.

Microbiology

- Bacterial cultures are performed on fresh tissue samples or swabs. Swabs in charcoal transport medium are the preferred specimens, as the charcoal medium allows the swabs to be cultured aerobically, anaerobically and for fungal culture. In addition, if there are likely to be delays in transport of specimens to the laboratory the charcoal transport medium suppresses the growth of any contaminants.
- Always remember to pack your culture specimens (swabs) in a separate sealed envelope from the histopathology samples, to avoid formalin fume contamination of the fresh specimens, which interferes with bacterial and fungal culture.

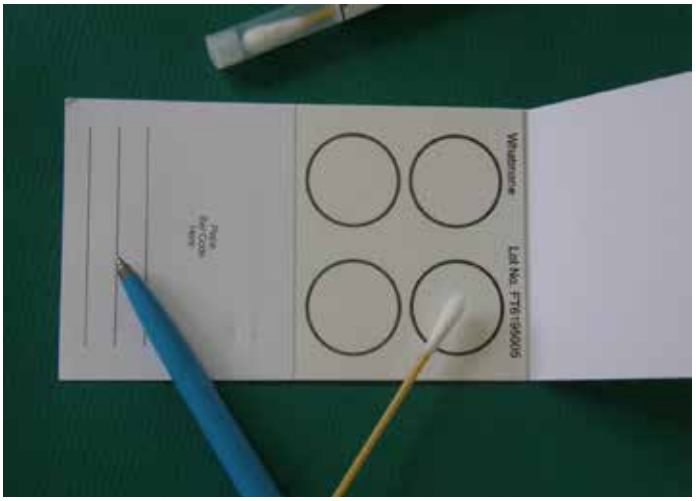

Figure 37. FTA cards.

Molecular Biology (PCR)

- The preferred samples for PCR analysis are dried swabs or FTA cards.

- FTA cards use special technology that simplifies the handling and processing of nucleic acids. Once the sample is applied to the card the nucleic acids are stabilised, so the sample does not have to be refrigerated and can be easily and safely transported to the laboratory.

- The sample can be applied by either rolling the swab onto the circular area of the card or from a small piece of organ tissue held in forceps and wiped or gently pressed onto the circular area.

- Repeat for subsequent samples until all 4 circles are filled, then identify the samples on the card (type, flock, age, house etc.), close the card and place in a plastic sleeve or zip-lock bag ready for sending to the laboratory.
- Fresh tissue specimens and EDTA blood samples are also suitable specimens.
- Swabs in transport medium complicate the nucleic acid extraction process and should be avoided.
- Heparin blood samples are contraindicated for PCR as the heparin anticoagulant interferes with the polymerase enzyme.

Bone Mineral Analysis

- From birds showing lameness, remove the leg at the hip joint to yield the femur, tibiotarsus and the tarsometatarsus (Figure 38).

- Cut away the flesh and tendons, etc., to leave relatively clean bone (still attached at each joint) (Figure 39).

- Clip off the foot (distal tarsometatarsus), place the bones from 5 legs into a plastic bag, place in the refrigerator, and then send to the laboratory on ice for bone mineral analysis (Figure 39).

Figure 38

Figure 39

- The resulting mineral levels can then be compared with standard normal levels for the same type of bird and similar age group.

References:

1. Abdul-Aziz T, Fletcher OJ, Barnes HJ. 2016. *Avian Histopathology*. 4th edition. American Association of Avian Pathologists, Florida.

2. Dinev I. 2010. *Diseases of Poultry: A Color Atlas*. 2nd edition. CEVA Sante Animal, Bulgaria.

3. Horner RF, Last RD. 2012. *VDX Poultry Health and Food Safety Manual*. Vetlink, Pretoria.

4. Saif YM. 2008. *Diseases of Poultry*. 12th Edition. Blackwell Publishing, Iowa.

Broiler Bone Analysis Guideline - Normal				
Age days	**% Ca**	**% P**	**% Mg**	**Ca:P**
0 – 14	32	17.5	0.8	1.8
15 – 28	33	17.9	0.6	1.9
29 - 42	34	18.0	0.6	1.9

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MULTIPLE-CHOICE QUESTIONS

QUESTION 1

Which of the following conditions is FREQUENTLY ASSOCIATED with linear proventricular haemorrhages?

a. Fowl typhoid
b. Newcastle disease
c. Fowl cholera
d. Mycotoxicosis
e. Salt poisoning

QUESTION 2

The ischiatic nerve is an important histological sample for the SCREENING of which of the following diseases?

a. Lymphoid leukosis
b. Avian encephalomyelitis
c. Mareck's Disease
d. Infectious bursal disease
e. Reovirus infection

QUESTION 3

Caecal tonsillar haemorrhages are an INDICATOR lesion for which of the following conditions?

a. Avian Influenza
b. Mesogenic Newcastle disease
c. Live vaccine reaction
d. Infectious bursal disease
e. Coccidiosis

QUESTION 4

What is the PURPOSE of examining growth plates in young broiler chickens?

a. Evidence of metabolic bone disease
b. Evidence of arthritis
c. Evidence of osteomyelitis
d. Signs of bone discolouration
e. Increased thickness of cortical bone

QUESTION 5

What is the EFFECT of vitamin D deficiency on the growth plate of young growing broiler chickens?

a. Narrowing of the zone of hypertrophy
b. Widening of the zone of hypertrophy
c. Hypertrophied cartilage zone of variable length
d. Narrowing of the zone of proliferation
e. Widening of the zone of proliferation

QUESTION 6

Anaemia of the bone marrow of young broilers would warrant the EXCLUSION of which of the following conditions?

a. Avian leukosis
b. Chicken anaemia virus
c. Chicken astrovirus
d. Reovirus
e. Mareck's disease

QUESTION 7

What is the TARGET tissue to 10% buffered formalin RATIO for ideal fixation for histopathology?

a. 1:1
b. 1:3
c. 1:5
d. 1:6
e. 1:9

QUESTION 8

Which is the PREFERRED sample type for bacterial culture?

a. Plane dried swab
b. Clear Amies medium swabs
c. Black charcoal medium swabs
d. Fresh tissues
e. Formalin fixed tissues

QUESTION 9

Which of the following specimen types would be CONTRA-INDICATED for polymerase chain reaction (PCR) analysis?

a. Dried swabs
b. FTA cards
c. EDTA blood sample
d. Heparin blood sample
e. Fresh tissue specimen

QUESTION 10

In broiler chicken bone analysis what is the TARGET calcium percentage for 14-day-old birds?

a. 17.5%
b. 0.8%
c. 35%
d. 18%
e. 32%

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