Postmortem procedure and diagnostic avian pathology

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\begin{abstract}
This review paper will highlight the basic systematic procedures involve from submission of carcass for the investigation of disease to diagnosis and will serve as a guide to veterinarians (anatomist and pathologist) on postmortem procedures in clinical practice.
\end{abstract}

\section{1. Introduction}

Necropsy (synonym: postmortem examination) is the systematic examination of a carcass with a view to searching for lesions that may point to the cause(s) of death. It is a very important diagnostic tool that is used to support other procedures performed in the diagnosis of disease conditions in man, a herd or flock of animals, birds, and fish/shell fish (Taiwo, 2005). There are several reasons for performing postmortem examination. These include: finding the cause of death, confirming a diagnosis, investigating unsuccessful therapy, increasing knowledge or satisfying curiosity. Diagnostic pathology is not limited to a necropsy. The pathologist uses the clinical history (including haematology, blood chemistry, and therapeutic measurements), the gross description,
culture results, and other data as well as the cytologic and histologic appearance of the lesions to make a diagnosis (Latimer, 1994). The rapidly expanding knowledge of animal diseases couple with diseases of zoonotic importance and to accommodate the interest of the owner necessitates the need for standard and improved veterinary diagnostic technics. This review paper will therefore highlight the basic systematic procedures involve from submission of carcass for the investigation of disease to diagnosis and also serve as a guide to veterinarians (anatomist and pathologist) on postmortem procedures.

2. Submission of carcasses or specimen

Submission of the intact carcass or sample from a group should be as soon as possible after death. Based on the necropsy findings, material will be collected and sampled for follow-up investigation. The quality of information received from such an examination is directly proportional to the quality and choice of the specimens submitted and the information that accompanies them. To promote rapid cooling of the carcass, thoroughly soak the plumage with cold water to which a small amount of soap or detergent has been added to aid complete wetting of the plumage and skin. Any feather that remains dry will provide insulation, which retards cooling of the carcass. Place the carcass in a plastic bag, squeeze out all excess air, seal or tie the bag, and refrigerate. The animal is kept refrigerated till the necropsy is done (Lowenstine, 1996).

In general, samples should be well identified (specie, age, sex, breed etc.) and cooled immediately upon death and be delivered to the necropsy room within 8 to 12 hours in our very hot tropical environment (Akpavie, 2004). It should be refrigerated (not frozen) and packed with sufficient ice or cool packs to keep it cold until arrival at the laboratory. If delivery to the laboratory is expected to be delayed beyond 96 hours, the carcass should be frozen immediately, rather than simply refrigerated. In instances where the carcass is extremely small, such as embryos, nestlings or very small adult birds, the entire carcass may be submitted for histologic examination. This is best accomplished by opening the body cavity, gently separating the viscera and fixing the entire carcass in 10% buffered formalin solution (Latimer, 1994).

3. Materials/equipments

It is pertinent that pathologists should be familiar with simple and useful set of instruments designated for postmortem examinations. These equipments should be aseptically cleaned thoroughly and sterilized after use. One should not use instruments that are used around living birds to avoid contamination of samples. Use adequate protective clothing; rubber gloves, rain boots, apron, face mask and eyes goggles. The instrument pack should contain forceps, 2 scapel handles (one for cutting, one for burning organ surfaces before taking a microbiology sample), necropsy knife, razor blade, stout scissors and/or poultry shears (for cutting bones), and fine scissors for dissection. Others include Strings for tying up hollow organs, small scale weighing balance, plastic ruler or measuring tape, aluminium foil, masking tape, labelling pens and gas burner for sterilizing equipments, small vails, sterile swab tubes, petri dishes, sterile syringes, specimen containers, clean and dry glass slides and coverslips for parasitological examination, hand-lens or dissecting microscope, and paper tissues. A digital camera will be useful for any gross lesions seen. A standard check list and necropsy report form will assist in recording observations.

| Table 1 | Tissues routinely collected for histopathology. |
|---|---|---|---|
| Skin | Thyroid gland | Rectum | Ovary and Oviduct |
| Feather follicle | Parathyroid gland | Caecum | Testes |
| Trachea | Oesophagus | Cloaca | Pectoral muscles |
| Lung | Crop | Spleen | Bone marrow |
| Air sac | Proventriculus | Liver | Thymus |
| Heart | Ventriculus | Gall bladder | Brain |
| Kidneys | Duodenum | Pancreas | Spinal cord |
| Adrenal glands | Small intestine | Cloacal bursa | Ischiatic nerve |

Source: Dorrestein, 1996.
Some reagents in solution are crucial: these include Cold normal saline solution (0.9% NaCl) for cleaning tissues/organs and parasitological examinations, 10% neutral buffered formalin (4% formaldehyde), 70% alcohol for wetting and disinfecting the feathers and skin, 80%, 95% and 100% (absolute) alcohol solutions for fixing specimens. Selection of additional tissues will depend upon gross lesions observed at necropsy (Graham, 1992).

4. Autopsy protocol (Anatomical approach)

There are probably many approaches to dissection in birds. Several procedures have been adopted and published depending on preference of pathologist. One should choose a procedure familiar and feels comfortable, use it consistently. Necropsy procedures are performed in a regular pattern and thorough manner, complete set of tissues and specimens be collected for subsequent histopathological, parasitological, toxicological, serological, and biochemical analysis to aid diagnosis. Carcass should be well disposed to avoid spread of disease to humans and animals.

The following procedure and checklist is used at the Necropsy Unit, Department of Veterinary Pathology, Usmanu Danfodiyo University Sokoto.

4.1. History

The history should attached with carcass; identification, physical findings, medical history.

Table 2

Summary of the most relevant data on work sheet.

<table>
<thead>
<tr>
<th>Clinic No</th>
<th>Date:</th>
<th>Post Mortem No:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinician’s Address:</td>
<td></td>
<td>Owner’s Name and Address</td>
</tr>
<tr>
<td>Species:</td>
<td>Breed</td>
<td>Age:</td>
</tr>
<tr>
<td>Number of animals in the Herd:</td>
<td>Number</td>
<td>Number Dead:</td>
</tr>
<tr>
<td>Number Affected:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Specimen(s) Submitted: |
| Indicate Below the Tissues Submitted and the Laboratory Reference Number: |

<table>
<thead>
<tr>
<th>Examination Required:</th>
<th>Bacteriology:</th>
<th>Virology:</th>
<th>Parasitology:</th>
<th>Photos:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Pathology:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histopathology:</td>
<td></td>
<td></td>
<td>Toxicology:</td>
<td>Others:</td>
</tr>
</tbody>
</table>

4.2. External examination and preparation of the carcass

Careful external examination should be made for the general body condition, weight, muscle mass, joints, dirty, ruffled feathers, localised tumor, and evidence of lice, mites, and pox lesion. Lice are best seen around cloacal area and beneath the wings, where their presence causes irritation of the skin and depluming. Evidence of diarrhea and of discharge from eyes, nose, or beak should be observed and recorded. Moistening or sprinkling the bird with water (preferably 70% alcohol) keeps down the dust and prevents the feathers from flying about during necropsy. The bird is positioned on its back (dorsal recumbency), in small birds the wings and legs are pinned to a dissecting board with nails or needles, large birds are fixed on a metal tray with pieces of rope. A table or box of suitable height will serve as a necropsy table and should be placed where there is adequate illumination by sunlight or direct light.

4.3. Opening the body cavities

The carcass can be grasped by both legs, which are pulled laterally to break the skin over the breast and the same time loosen the coxofemoral joints. This will enable the bird to lie flat on its back and the cut skin serves as the first point of incision. The skin and feathers are removed by digital dissection from beak to the anus and from the sides of the neck and body. The condition of the breast muscle is observed for loss of muscle mass; hemorrhage and the subcutaneous tissue are also noted.
A sharp knife or scissors will suffice to do complete necropsy in birds, although plain or rat-toothed forceps and bone forceps will aid in a clean necropsy procedure. A straight line incision is made through the abdominal wall across the posterior abdominal region. Incision is extended on either side of the abdomen and forward along the rib articulations, and then the breast can be removed, exposing both the abdominal and pleural cavities. Observe the presence of fluids, exudate, or blood in either cavity. The visceral contents are examined in situ for any gross lesion before they are removed.

4.4. Examination of the organs

This begins by cutting gastrointestinal tract at the termination of the esophagus, the proventriculus, gizzard, the pancreas which rest on duodenal loop, and the large intestine including cecae are removed. The liver and spleen are removed together.

In birds the kidneys are lobulated and closely attached with bony structure of the sublumber region. They extend from the ovary or testes to the cloaca. Discolored or mishapen structures are indicative of abnormality or a disease. The oviduct need not to be removed but can be examining in situ. It may contain exudate which can be seen externally as a discharge from the cloaca, or observe only upon dissecting the oviduct.

The heart and lungs are usually examined in situ but may be removed when detailed examination is required. Normal avain heart is like a cone shaped, assess colour, the thickness of the ventricle walls. Lungs are pink in colour and do not collapse until their attachments are broken. The air sacs, present in both thorax and abdomen, are formed by a thin layer membrane normally unnoticed unless filled with exudate, when it appears cloudy or covers an accumulation of whitish yellow exudate.

The positioning is change so that the head is directed toward the examiner. With the aid of knife or scissors a cut is made through the uppermost commissure of the beak. The incision is continued through the oral cavity and throat, down the esophagus and through the crop to the point where the gastrointestinal tract was previously cut. When inspection is done, cut through the larynx to open the trachea throughout its length, followed by the bronchi into the lungs.

The carcass is then placed on ventral recumbency with the skin and feathers are detached from the back region to permit examination of the brachial plexus between the scapula and the vertebral column. After, the sciatic nerve is exposed by separating muscles of the leg with the bird placed on its back. Enlargement of the branchial or lumbosacral plexus of the sciatic nerve is an indication of certain viral disease like Marek's.

Alimentary tract is the last to be examined to avoid contamination, open the proventriculus for inspection of the mucosa and the glands. The opening is extended through the gizzard and its muscles. Intestine is opened along its entire length; look out for its contents, presence of parasites, and the condition of the mucus membrane. Open the cecum and examine the contents. Check for prolapse, inflammation, and other disoders of the cloaca.

5. Conclusion

Careful postmortem examination supported by one or more antemortem laboratory investigations can greatly assist in arriving at confirmatory diagnosis. Diagnostic poultry pathology involves a multidisciplinary approach to many poultry diseases. Ranging from histopathologist, clinical pathologist, toxicologist, microbiologist, virulogist and parasitologist.

References


