

Diagnostic Considerations for Marek's Disease

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The control of Marek's disease by vaccination was a key event in the expansion poultry production worldwide. Continued success in controlling Marek's disease will be part of the effort to double global food production by 2050. Control of Marek's disease is a two-phase problem involving 1) accurate diagnosis, and 2) applying appropriate procedures to prevent the disease. This presentation offers practical guidelines for diagnosis or exclusion of Marek's disease as a differential diagnosis in chickens in commercial production.

Marek's disease and other tumor diseases of chickens present a unique diagnostic challenge. The gold standard for diagnosticians in the field and laboratory is the new monograph, *Tumor Diagnosis Manual: The Differential Diagnosis of Lymphoid and Myeloid Tumors in the Chicken* (Witter et al. 2010). Additional new information can be found in *Frequently Asked Questions about Tumor Viruses* (AAAP Tumor Virus Committee, 2012). Lymphoid and myeloid tumors occur commonly in all breeds of chickens and are economically important to commercial poultry management. Accurate identification of the disease requires pathological and etiologic assessment of tumors caused by three or more viruses, potentially multiple viral infections in the same chicken, and sorting through confounding inflammation. The field diagnostician is responsible for obtaining a history, characterizing the epidemiology and clinical observations, documenting the gross pathology, and collecting specimens for the laboratory.

Broilers. In the USA, Marek's disease becomes a concern in broilers when skin lesions are identified at slaughter inspection, which results in condemnation of the entire carcass. A prompt and accurate diagnosis is critical to resolve the issue, which may involve a newly emerging virus strain, vaccine failure for various reasons, or a training deficiency in an inspector –problems with very different solutions. Broilers can be condemned at slaughter for lymphomas in visceral organs, especially in older broilers raised for further processing. Skin lesions may occur in broilers with lymphoma.

The classic Marek's disease paralysis syndrome is uncommon today in broilers but can occur with errors in vaccination or the emergence of a new virus strain. Transient paralysis syndrome occurs in production and must be differentiated from other disease of the central nervous system including botulism, intoxication, and infectious disease. The impact of the early lymphocytic phase of Marek's disease on flock immunity is not well understood and deserves further study in the commercial environment. Some programs that use herpesvirus-vectored vaccines for bursal disease or laryngotracheitis, actually monitor for subclinical Marek's disease as an indicator of vaccine efficacy using histopathology. Nerve, gonad, skin and eye from broilers of processing age are examined along with bursa and thymus.

The follow responsibilities are assigned to the field diagnostician when facing a potential problem of Marek's disease or other tumors in broilers.

1. History and Epidemiology. Obtain a complete history and characterize the epidemiology;
 - a. Age of birds affected; date first observed; breeder flock and hatchery source; farm history
2. Necropsy. Examine multiple birds at necropsy, recording the incidence and severity of lesions, being certain to examine skin, viscera, and nerves.
 - a. Photograph representative lesions – cell phone cameras are adequate.

3. Histopathology. Collect tissue in 10% neutral buffered formalin for histopathology from 3 to 5 typical chickens
 - a. Skin, selecting typical lesions leaving about 1-cm margins
 - b. Nerves from each chicken, to include the vagus, brachial plexus, and sciatic nerve. Select enlarged nerves if possible.
 - c. Obvious tumors; also spleen, liver, kidney, gonad, brain, and proventriculus from each chicken
 - d. One eye from each affected chicken (not always possible).
4. Virus detection. Requires FTA cards and Whirl-Pak bags in the necropsy kit.
 - a. If possible, identify a live broiler with skin lesions, euthanize, and collect tissues. Also collect tissues from processed broilers.
 - b. Blot the incised surface of the enlarge follicle on FTA paper; also blot the feather pulp if available. See AAAP tumor virus committee report for instructions on collecting feather pulp.
 - c. Tumors. If obvious tumors, cleanly incise a piece from the center of the tumor and blot on FTA card. Clearly label the card to indicated individual tumor and feather pulp identity.
 - d. Collect feather pulp, skin lesions, and tumors in separate Whirl-Pak bags, place on ice for transport, and then freeze, pending the need for further testing. If possible, store in a freezer that does not automatically defrost, or on dry ice (CO₂), or liquid nitrogen.

Breeders and Layers. In my experience, the practical challenges of tumor diagnosis in breeders and layers are as follows.

- 1) Is the lesion observed at tumor or a non-neoplastic lesion?
- 2) Is it a lymphoma, and if so, is it Marek's disease, avian leukosis, reticuloendotheliosis, or a spontaneous lymphoma with no viral association?
- 3) If the tumor is other than lymphoma, and can it be linked to a viral cause, such as one of the avian leukosis viruses.

Each situation involves very different prevention and control strategies, and the added potential of business and legal implications. A critical step is the collection of the correct samples that can lead to an accurate diagnosis. All other responses to the problem proceed from this critical information.

The follow responsibilities are assigned to the field diagnostician when facing a potential problem of Marek's disease or other tumors.

1. History and Epidemiology. Obtain a complete history and characterize the epidemiology;
 - a. Age of birds affected; date first observed; breeder flock and hatchery source; farm history
2. Clinical examination. Select sick chickens for euthanasia and necropsy. The enlarged bursa of Fabricius can be palpated with a gloved finger on through the dorsal wall of the cloaca. Enlarged bursas may have lymphomas of diagnostic value.
3. Necropsy. Examine multiple birds at necropsy, recording the incidence and severity of lesions, being certain to examine skin, viscera, bursa of Fabricius, thymus, nerves, and eyes.
 - a. Photograph representative lesions – cell phone cameras are adequate.

4. Histopathology. Collect tissue in 10% neutral buffered formalin for histopathology from. Collect tissues from daily mortality chickens and from euthanized cull chickens suspected to have the disease.
 - a. Skin, selecting feather follicle lesions.
 - b. Nerves from each chicken, to include the vagus, brachial plexus, and sciatic nerve. Select enlarged nerves if possible.
 - c. Obvious tumors; also spleen, liver, kidney, gonad, thymus, bursa of Fabricius, brain and proventriculus from each chicken
 - d. One eye from each chicken.
5. Virus isolation and detection. Requires FTA cards and Whirl-Pak bags in the necropsy kit.
 - a. Collect tissues from euthanized birds or from the most recent mortality.
 - e. Blot the incised surface of the enlarge feather follicle on FTA paper; also blot the feather pulp. See AAAP tumor virus committee report for instructions on collecting feather pulp.
 - b. Tumors. If obvious tumors, cleanly incise a piece from the center of the tumor and blot on FTA card. Label the card.
 - c. Collect feather pulp, skin lesions, and tumors in separate Whirl-Pak bags, place on ice for transport, and then freeze, pending the need for further testing. If possible, store in a freezer that does not automatically defrost, or on dry ice (CO₂), or liquid nitrogen.

Preservation of cellular detail is important for classifying tumors by histopathology; however, cellular detail rapidly declines after death. Examination of recent mortality, or finding affected sick birds for sampling is therefore important.

FTA cards provide the option of qualitative and quantitative molecular detection (PCR) tests. Marek's disease diagnostic confirmation and tumor virus diagnostics in general actually depends as much on negative tests as positive tests for the various tumor viruses. Once negative test results for retroviruses are received, the Marek's virus testing can become more focused as to the serotype and strain present.

Some virus detection work can be done using paraffin-embedded tissues from histology. Marek's disease virus can be detected in most cases because chickens are vaccinated and the virus infection persists for life. Marek's disease tumors yield substantially more viral DNA, and this can be assessed by quantitative real-time PCR. The presence or absence of avian leukosis virus and reticuloendotheliosis virus is therefore significant when aligned with the histopathology and other molecular detection data.

The collection of frozen fresh tissues for virus isolation from field cases is not ideal because standard freezers are not cold enough to stop the titer decline of viable virus. Storage on dry ice or liquid nitrogen is better, but recognize that virus viability may rapidly decline under field conditions.

The ability to classify tumors by cell surface antigens and immunohistochemistry requires collection in special embedding media (O.T.C.[®]). This work is done at special reference laboratories and requires specific instructions for collection and transport of specimens. General instructions are available at the AAAP FAQ tumor virus site. In a similar manner, the collection of white blood cell "buffy coat" preparations in dimethyl sulfoxide (DMSO) from heparinized blood samples requires consultation with the reference laboratory. The procedures are not detailed here because they generally used as necessary at a more advanced stage of the investigation.

In summary, an effective response to a potential problem of Marek's disease first requires a definitive diagnosis. Diagnosis and response depend on an accurate history, epidemiological assessment, and the correct samples for pathology and virus testing. Conversely, the omission of the proper samples can lead to inconclusive diagnostic testing and delays in developing an effective control response to Marek's disease or other viral tumor problem.

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References

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AAAP Tumor Virus Committee. Frequently asked questions about tumor viruses. 2012. <http://www.aaap.info/frequently-asked-questions-on-viral-tumor-diseases>