Title: Marek’s disease in a Buff Silkie chicken

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Microscopic Findings: The cutaneous mass was a non-encapsulated, poorly demarcated, infiltrative, densely cellular neoplasm composed of round cells arranged in sheets with fine fibrovascular stroma (Figure 2A). Neoplastic cells had distinct cell borders with scant amphophilic homogeneous cytoplasm and round centrally located nuclei with coarsely to hyperchromatic chromatin and inconspicuous nucleoli (Figure 2B). Anisokaryosis and anisocytosis were mild to moderate; mitotic figures were rare (less than 1/10 hpf). Rare tingible-body macrophages were mixed with the neoplastic cells. The coelomic mass was composed of similar neoplastic round cells, which infiltrated into the adjacent kidneys as well as the sciatic plexus and the surrounding connective tissue (Figure 2C and 2D). Other organs effaced by similar neoplastic round cells include lungs, heart, gastrointestinal tract, pancreas, ovary, oviduct, and bone marrow.

Morphologic Diagnosis: Skin, kidneys, peripheral nerve, lung, heart, gastrointestinal tract, pancreas, ovary, oviduct, and bone marrow: Lymphoma

Etiology: Gallid herpesvirus type 2

Disease: Marek’s disease

Discussion: Marek’s disease (MD) is a viral induced lymphoproliferative disease of chickens with a predilection for peripheral nerves, various visceral organs, skin, and iris. Gallid herpesvirus type 2 (serotype 1), the etiologic agent of MD, belongs to the Alphaherpesvirinae subfamily and the Mardivirus genus. Gallid herpesvirus type 2 has further been categorized into mild (m) MDV, virulent (v) MDV, very virulent (vv) MDV, and very virulent plus (vv+) MDV strains. Avian species including chicken, quail, turkey, pheasant and some species of waterfowl have been recorded to be susceptible to MDV infection; among these, chickens have been considered the most important natural host for MDV, getting to develop disease. MD can occur beginning at 3-4 weeks of age, although it is not limited to young birds. Inhalation of contaminated feathers, dander, and dust containing Marek’s disease virus (MDV) leads to disease initiation whereas vertical transmission of the virus is unlikely. Four phases of MDV infection have been demonstrated: (1) Early productive-restrictive virus infection, (2) Latent infection, (3) Second phase of cytopolytic productive-restrictive infection, and (4) Proliferative phase. Once the respiratory system is exposed to MDV, phagocytic cells, mostly macrophages, transfer the virus.
to lymphoid organs, which leads to cytolytic responses predominantly in B cells, contributing to lymphoid depletion and immune suppression, followed by neoplastic transformation of primarily T cells.\textsuperscript{3,4}

Figure 2. Chicken, Lymphoma in the caudodorsal skin (A and B), kidney and sciatic plexus (C), and sciatic plexus (D). A: Expanding and replacing the dermis is a non-encapsulated, poorly demarcated, infiltrative, densely cellular neoplasm. H&E, 10x. B: Neoplastic round cells are arranged in dense solid sheets and have distinct borders, scant cytoplasm, and a single round nucleus. H&E, 40x objective. C and D: Similar neoplastic round cells infiltrate and partially replace the kidney (C: asterisk) and sciatic plexus (C: arrows; D). H&E, 5x and 10x, respectively.

Clinical signs associated with MD depend on the location where the lesions evolve. Neoplastic cell infiltration of the vagus nerve can lead to dilation and paralysis of the crop; sciatic nerve and sciatic plexus involvement most often presents with the characteristic signs of unilateral paresis or paralysis of the legs and one leg stretched forward; and when the eyes are involved, chickens develop blindness and reduced pupillary light reflex. Affected chickens may therefore be unable to reach their food and water, resulting in starvation, dehydration and even death. Other non-specific signs include weight loss, anorexia and diarrhea.\textsuperscript{5}

Gross findings with MD consist mainly of visceral and neural lesions. Integumentary and ocular changes have also been reported. Visceral lymphoma, which may affect different organs, has been associated with virulent strains of MDV. Affected organs contain soft to slightly firm, variably sized, white to gray nodules that extend into the parenchyma and replace the normal tissue structure. In the neural form, the affected peripheral nerve loses the cross striations and presents segmental or diffuse enlargement, with gray or yellow discoloration and occasionally edematous appearance. The skin lesion is characterized by whitish nodules scattered mostly around feather follicles. In association with infiltration of the iris by neoplastic cells, chickens have loss of iridal pigmentation with gray discoloration and irregularity of the pupil.\textsuperscript{3,5}
With MDV infection, there are three types of histologic lesions in the peripheral nerve. One type is termed Type A, which is recognized as a neoplastic form, composed of pleomorphic lymphocytes, including lymphoblasts, and large, medium, and small lymphocytes. The second type is Type B, which consists of inflammatory cells, including mainly small lymphocytes and plasma cells, and is frequently accompanied by edema. The last type is Type C, which has similar lesions as in Type B except for reduced severity and absence of edema. Lesions in the visceral organs are of similar composition as the neoplastic form, Type A, in the affected peripheral nerve. Lesions in the integument are located primarily around the feather follicles. Iris lymphoma, which renders chickens to lose the ability to maintain the normal size and shape of the pupil, is characterized by neoplastic cell infiltration of the iris.3,5

Differential diagnoses of lymphoma in chickens include two retroviral diseases in addition to MD. One is avian lymphoid leukosis and the other is reticuloendotheliosis. In the present case, lymphoma was seen in multiple organs including in the peripheral nerve and skin while there was no involvement of the bursa of Fabricius. This constellation of lesions is characteristic of MD. Confirmatory ancillary tests for the diagnosis of this disease are virus isolation, polymerase chain reaction (PCR) assay and in situ hybridization (ISH) to detect viral nucleic acid and enzyme-linked immunosorbent assay (ELISA), virus neutralization (VN) testing and immunohistochemical staining (IHC) for viral antigen detection.4,5

Adoption of vaccination is the strategy for MD prevention and control. Aside from low pathogenic strains of Gallid herpesvirus type 2 (serotype 1), two other herpesviruses - Gallid herpesvirus type 3 (serotype 2) and Meleagrid herpesvirus 1 (previously called Turkey herpesvirus [HVT], serotype 3) - have been widely used in the production of individual as well as bivalent serotype 2+3 commercial vaccines. In ovo vaccination of chicken embryos is the most common route used nowadays at 17-19 days of incubation. An alternative is subcutaneous vaccination of 1-day-old chicks, which has been recognized as an additional effective and significant way to diminish morbidity and mortality by MD.1,2

References:

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