Pathology in Practice

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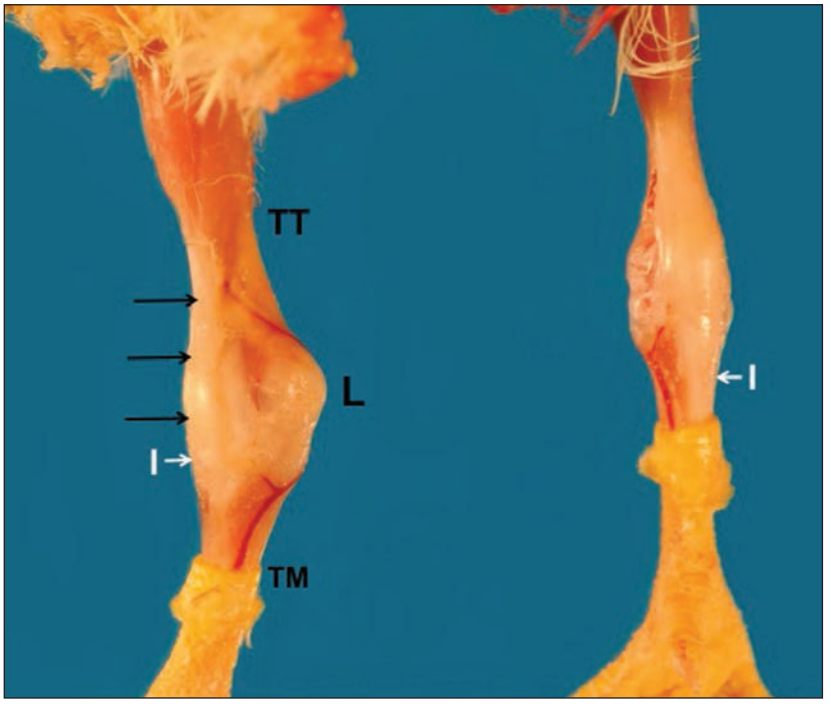

Figure 1—Photograph of the plantar aspect of the legs of a 3-week-old chick *(Gallus gallus domesticus)* with an apparent broken leg. The left tibiotarsometatarsal (hock) joint region (L) is swollen, the gastrocnemius tendon is deviated laterally (black arrows), and the leg is rotated approximately 45° outward. The insertions of the gastrocnemius tendons (I) are indicated with white arrows. TM = Tarsometatarsus. TT = Tibiotarsus.

History

A 3-week-old unvaccinated chick (*Gallus gallus domesticus*) from a research flock was reported to the clinical veterinarian because of a possible broken leg. Palpation failed to reveal a fracture. The chick was euthanized by carbon dioxide asphyxiation and was submitted for immediate necropsy. Although it was not known at the time of necropsy, the chick was from the Regional Poultry Research Laboratory line 7.1.

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Clinical and Gross Findings

The chick weighed 107.5 g (0.24 lb) and had both primary and secondary feathering of the wings with down on its head and body. Its postmortem condition was excellent. The region of the left tibiotarsometatarsal (hock) joint was swollen (14 mm in width, extending 6 mm proximally and 14 mm distally); the right hock joint region was 10 mm in width (extending 4 mm proximally and 10 mm distally). Removal of the overlying skin revealed lateral deviation of the left gastrocnemius tendon (**Figure 1**), colloquially referred to as perosis. A 9 × 9 × 8-mm pale pink mass with a granular surface was present in the mucosa at the esophageal-proventricular junction. There were no other gross lesions of bones or viscera identified at necropsy.

Formulate differential diagnoses from the history, clinical findings, and Figure 1—then turn the page →

Histopathologic Findings

Samples of various tissues were placed into neutral-buffered 10% formalin, processed with routine paraffin embedding, and stained with H&E stain for histologic examination. There was mild synovial hyperplasia of the left hock joint; the gastrocnemius tendon was not present in parasagittal section as a result of antemortem displacement. Chondrodystrophy was ruled out by histologic examination of decalcified sections of both tibiotarsometatarsal joints.

Within the tissues of the proventriculus (near the esophageal junction), duodenum, ceca, spleen, kidneys, skin, and feather follicles were poorly defined, densely cellular, infiltrative, and unencapsulated masses comprised of cells arranged in closely packed sheets separated by minimal fibrovascular stroma. Neoplastic cells were medium-sized to large, pleomorphic (but typically round), and often lymphoblastic, with indistinct cell borders, small quantities of homogenous pale eosinophilic cytoplasm, and a large ovoid central nucleus with finely stippled chromatin and a poorly apparent magenta nucleolus (**Figure 2**). Moderate anisocytosis and minimal anisokaryosis were evident. There was a mean of 1.9 mitotic figures/400X field (range, 0 to 4 mitotic figures/400X field). Many necrotic cells were noted.

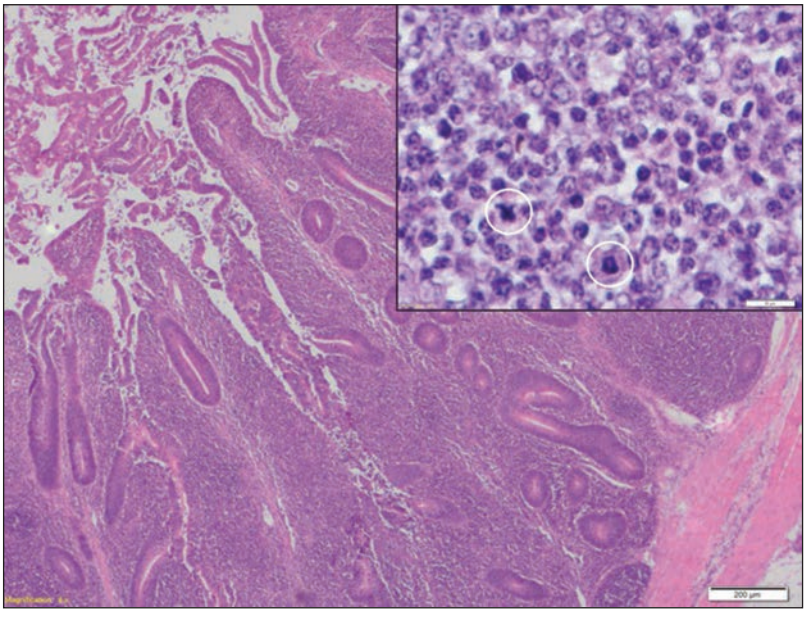

Figure 2—Photomicrographs of a section of the cecum of a 3-week-old Regional Poultry Research Laboratory line 7.1 chick. Notice the infiltration of the lamina propria by a densely cellular mass, which widely separates the glands. H&E stain; bar = 200 µm. Inset—The mass is composed of pleomorphic neoplastic lymphocytes and lymphoblasts with 0 to 4 mitoses/400X field. Two mitotic figures are circled. H&E stain; bar = 10 µm.

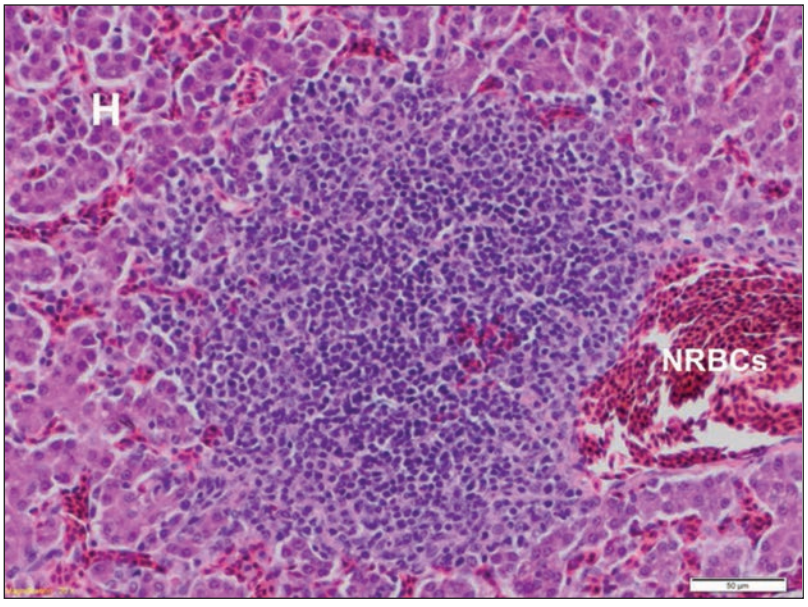

Figure 3—Photomicrograph of a section of the liver of a flockmate of the chick in Figure 1, which was euthanized and necropsied at 5 weeks of age. Notice a focus of lymphomatous infiltrate within the parenchyma. H = Hepatocytes. NRBCs = Nucleated RBCs. H&E stain; bar = 50 µm.

Morphologic Diagnosis and Case Summary

Morphologic diagnosis: unilateral lateral deviation of the gastrocnemius tendon with mild synovial hyperplasia (perosis) of the left tibiotarsometatarsal joint and multicentric lymphoma of the proventriculus, duodenum, ceca, spleen, kidneys, skin, and feather follicles.

Case summary: perosis and Marek's disease in a chick.

Comments

The lesions identified in the chick of this report were indicative of 2 disease processes: one nutritional and the other infectious. The gross identification of a slipped gastrocnemius tendon was strongly suggestive of nutritional deficiency, and the common causes of perosis include deficiencies in manganese, choline, pantothenic acid, biotin, niacin, and folic acid.[1,2] Histopathologic changes consistent with manganese deficiency (chondrodystrophy characterized by decreased numbers of disorganized chondrocytes in the zone of proliferation, decreased extracellular matrix, and widening of the physis) and choline deficiency (hyperkeratosis) were not identified in the affected chick. There was no gross or histologic evidence of hemorrhage or inflammation (suggestive of avian reovirus or *Mycoplasma synoviae* infection). Genetic causes are plausible, especially in an inbred line, but poor formation of hock joints with slipped gastrocnemius tendons in the Regional Poultry Research Laboratory line 7.1 has not been reported in the 37-year period since its establishment.[3] In support of a nutritional cause, chicks of other lines from 2 other facilities were subsequently reported as having curled toes, suggesting riboflavin deficiency. There was no evidence of slipped gastrocnemius tendons in these chicks. The chief differential diagnoses for the

slipped gastrocnemius tendon and curled toes observed in all 3 research flocks were deficiencies in pantothenic acid, biotin, folic acid, niacin, or riboflavin; multiple deficiencies were likely. The exact vitamin deficiencies were not determined. Further inquiries about the feed determined that an outdated vitamin and mineral premix had been used to formulate the ration fed to all 3 groups of chicks. Use of an up-to-date vitamin and mineral premix was implemented, and no further cases of slipped gastrocnemius tendon or curled toes have been reported.

The other major disease process in the chick of this report was lymphoma. Based on the age of the bird and the distribution of the tumors (ie, sparing of the bursa of Fabricius), a diagnosis of Marek's disease was suggested. Molecular confirmation of infection with Marek's disease virus via PCR assay was not pursued because of cost. Clinical signs of Marek's disease can appear as early as 3 to 4 weeks after infection; signs are grossly and histologically detectable 2 to 4 weeks after infection.[4] The disease is often associated with unilateral progressive paresis of a leg in chicks between 6 and 10 weeks of age (after exposure to the virus at hatching); the paresis is attributable to lymphomatous infiltration of the sciatic nerve. Typical gross lesions of Marek's disease include enlargement of the peripheral nerves (especially the sciatic nerve) with gray or yellow discoloration and loss of striations. Additionally, visceral lymphomas are common and involvement of the iris with gray discoloration is a classic lesion. However, there was no clinical, gross, or histologic evidence of neurologic or ocular disease in the 3-week-old chick of this report.

Approximately 2 weeks after infection of chickens with the alphaherpesvirus that causes Marek's disease, excretion of infective virus ensues; it peaks between 3 and 5 weeks after infection, and infected chickens shed virus indefinitely.[4] Viral replication occurs in feather follicle epithelium, and virions are shed in feather dander. Feather dust is infectious for 4 to 8 months at room temperature (approx 20° to 23°C).[4] Other birds inhale the shed virions, which initially infect B lymphocytes and result in cytolysis. Secondary infection of T cells can occur, causing neoplastic transformation (lymphoma).[4] There has been recent advancement in the understanding of this T-cell transformation. Within Marek's disease virus–infected T cells, there is rapid downregulation of HIC1 transcription factor activity, which leads to a loss of cellular tumor suppressor mechanisms.[5] Several days later, the Marek's herpesvirus oncogene *Meq* is expressed, promoting tumor development.[6,7]

Given the diagnosis of Marek's disease for the chick of this report, 10 other chicks from the same unvaccinated group were euthanized at 5 weeks of age, and pooled tissues were examined histologically. At least 6 chicks were confirmed to have lymphoma in the liver (**Figure 3**), spleen, or ceca. Lymphoma was not identified in the bursae of Fabricius or the sciatic nerves of any of those birds. Lymphoma in the bursa of Fabricius is much more commonly associated with avian leukosis retrovirus infection; in these cases, it typically develops in infected chickens > 14 weeks of age. In addition to lymphoproliferative disease, Marek's disease virus can also cause degeneration and inflammation of peripheral nerves, demyelination and vasogenic edema in the CNS and peripheral nervous system, immunosuppression (via thymic and bursal atrophy), atherosclerosis, and decreased egg production in infected poultry.[4] There is evidence that increased circulating concentrations of corticosteroids may precipitate development of Marek's disease in chickens following infection,[8] and it is possible that the severity of the disease in the chick of this report was influenced by the concomitant nutritional deficiencies. Infection with the Marek's disease herpesvirus exacerbates intercurrent infections, and coinfections with immunosuppressive viruses such as those causing infectious bursal disease, reticuloendotheliosis, and chicken infectious anemia are characterized by increased severity of both diseases.[4]

The Regional Poultry Research Laboratory line 7.1 is an inbred line of chickens with homozygosity at the B^2 allele of the major histocompatibility complex. Chickens of this line are known for their susceptibility to Marek's disease.[9] Regional Poultry Research Laboratory line 7.1 chickens were used in the development of vaccines against Marek's disease.[10] Recent research into the genetic causes of variable susceptibility among chickens to Marek's disease has identified IRG1 polymorphism as a factor in disease susceptibility.[5]

A vaccination program for protection against Marek's disease and diseases associated with other common chicken pathogens was developed and implemented for the research flocks; no further cases of lymphoma have since been identified. Development of a vaccine against Marek's disease was the first demonstration that vaccines could prevent cancer.[4] The case described in this report underscores the importance of complete necropsy and histologic evaluation of selected tissue samples in the identification and prevention of disease in a flock.

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