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What Is Your Diagnosis? Peripheral neuritis secondary to Marek's disease.

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## What Is Your Diagnosis?

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## What Is Your Diagnosis?

### History

A 14-month-old, male black and red Shamo chicken was presented to the Avian and Exotics Service, Ontario Veterinary College Health Sciences Centre (Guelph, Ontario, Canada) with a 3.5-week history of left pelvic limb lameness. The owner initially noticed the bird to be “limping,” with the left leg affected. The ability of the chicken to walk continued to decline, and the bird had to be isolated from the rest of the flock. A local poultry veterinarian evaluated the bird and prescribed an antibiotic in the drinking water (spectinomycin, 0.5 g/L) but did not establish a diagnosis for the lameness. The lameness appeared to stabilize, but no improvement to the patient’s condition was observed by the owner.

The bird was housed outdoors in a coop with free-range access, along with 30 other chickens. The birds were fed a diet of formulated grower crumble with some grain and fresh tap water provided on a daily basis. The flock was dewormed annually with ivermectin (0.2 mg/kg orally), but

vaccination history was unknown. All other birds in the flock appeared healthy, although one hen in the flock had recently died a few days after hatching its eggs.

On physical examination, the chicken was bright, alert, and responsive, with voluntary movement in all limbs, but appeared unkempt. Severe nonweight-bearing lameness with a plantigrade stance was noted in the left pelvic limb (Fig 1). Despite the functional deficit involving the left leg, the bird was ambulatory without assistance. On examination of the affected limb, no palpable fracture or luxation was appreciated, but severe muscle atrophy was evident (Fig 2). A neurologic examination revealed a decreased flexor reflex in the left pelvic limb and absent patellar reflexes bilaterally. The remainder of the neurologic examination and external physical examination were unremarkable.



**Figure 1.** A backyard chicken that presented for left pelvic limb lameness.



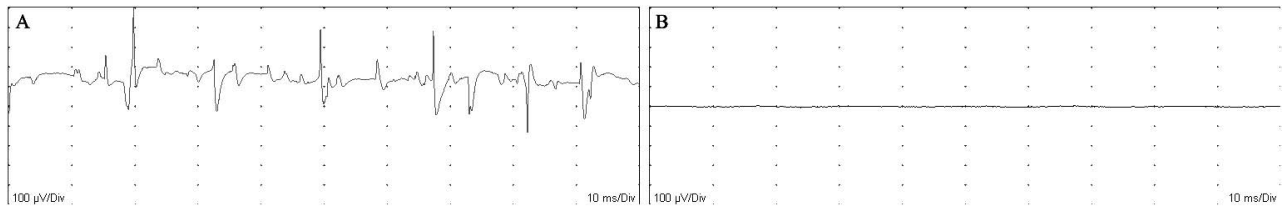
**Figure 2.** Anterior view of the thighs of a backyard chicken that presented for lameness. Note the significant muscle atrophy of the left pelvic limb (L) compared with the normal right pelvic limb (R).



**Figure 3.** Placement of electromyogram electrodes in the muscles of the right pelvic limb of the chicken described in Figure 1 with left pelvic lameness.

The chicken was anesthetized with isoflurane gas, and whole body radiographs were obtained; no abnormalities were detected. An electromyogram (EMG) was performed by inserting a concentric needle electrode (Technomed Europe, Maastricht-Airport, the Netherlands) along with a subcutaneous needle electrode (Ambu Neuroline; Ambu Inc, Columbia, MD, USA), used as a ground, into various muscle groups of each pelvic limb (Fig 3). Electrical activity was monitored on a Cadwell Sierra Wave machine (Cadwell Laboratories, Inc, Kennewick, WA, USA). Prolonged insertional activity, spontaneous fibrillation potentials, and runs of positive sharp waves were found in the left gastrocnemius, iliofibularis, long digital extensor, and some flexor muscles (Fig 4). The left flexor cruris lateralis and all corresponding muscles of the right pelvic limb showed normal EMG activity.

After discussion with the client, the decision was made to euthanize the chicken. The body was submitted for postmortem examination.



**Figure 4.** Comparison of electromyogram (EMG) findings between (A) the affected left and (B) the normal right lateral gastrocnemius muscles in the chicken with left pelvic limb lameness described in Figure 1. Moderate fibrillation potentials with interspersed positive sharp waves are seen in the EMG of the left lateral gastrocnemius.

*Please evaluate the history, physical condition findings, and Figures 1–4. Formulate a list of differential diagnosis and consider other diagnostic tests before proceeding.*

### Diagnosis

Based on the signalment, clinical signs, and EMG findings, the tentative diagnosis was peripheral neuritis secondary to Marek's disease (gallid herpesvirus 2). Differential diagnoses included different types of neoplasia (eg, peripheral nerve sheath tumor), avian leukosis virus, trauma, or vascular compromise.

On postmortem examination, the bird appeared adequately hydrated and in good body condition, with appropriate fat stores. Severe, diffuse, unilateral atrophy of multiple muscle groups of the left pelvic limb was identified. Leg measurements taken immediately distal to the left and right stifle joints, in the area of the anterior tibialis and gastrocnemius muscles, revealed circumferences of 17 cm and 21 cm, respectively. In addition, a well-demarcated, raised, 5-mm diameter, white-tan nodule was identified on the surface of the right cranial liver lobe.

Histopathologic examination of multiple sections of skeletal muscle from the left thigh revealed increased amounts of fibrous connective tissue within the perimysium and diffuse variation in myofiber size (Fig 5). Within individual fascicles, contiguous groups of hyper eosinophilic, small, angular fibers were present, in addition to multiple swollen hyper eosinophilic fibers that often contained large vesicular nuclei and displayed sarcoplasmic vacuolation. Rarely, swollen, debris-containing mononuclear cells (likely macrophages) were observed throughout the sections. Within the left ischiadic nerve, increased numbers of lymphocytes and plasma cells were present in the endoneurium, in addition to axonal swelling and myelin degeneration (Fig 5). Occasional dilated myelin sheaths contained myelin debris and macrophages. Mild, multifocal, lymphoplasmacytic infiltrates were also identified within the right ischiadic nerve, left and right brachial nerves, myenteric plexus, and iris.

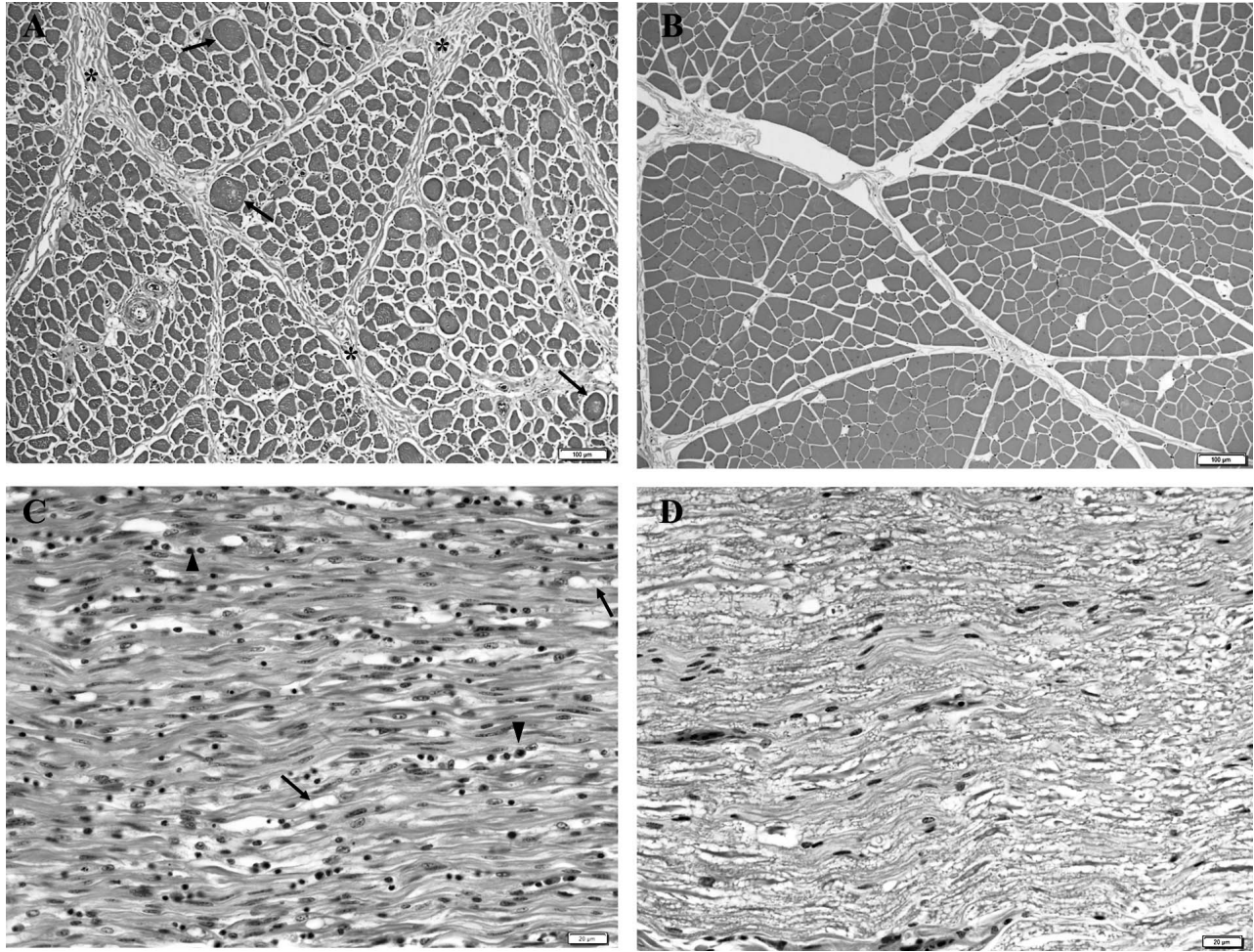
Histopathologic examination of the liver nodule revealed an unencapsulated, relatively well-circumscribed yet locally infiltrative, densely cellular mass of polygonal cells forming small nests and poorly arranged tubules among an abundant mature collagenous stroma. These cells were hepatoid in appearance, with distinct cell borders and abundant, deeply eosinophilic to basophilic, finely granular cytoplasm. The polygonal cells contained a single central, round to oval nucleus with coarsely stippled chromatin and inconspicuous nucleoli and displayed up to threefold anisocytosis and anisokaryosis with 4 mitotic figures observed

in ten  $\times 400$  high-power fields. Immunohistochemical staining was not performed, and the mass was deemed most consistent with a carcinoma of hepatic origin accompanied by an overwhelming scirrhous response; however, a nonneoplastic inflammatory process could not be ruled out.

The identification of a lymphoplasmacytic polyneuritis, in conjunction with unilateral muscular lesions consistent with neurogenic atrophy, was deemed highly suggestive of Marek's disease. To pursue a definitive diagnosis, a paraffin block containing left ischiadic nerve, as well as frozen tissue samples of the heart, liver, lung, kidney, and spleen were submitted for polymerase chain reaction (PCR) testing (Service de diagnostic at the Faculté de Médecine Vétérinaire—Université de Montréal, Quebec, Canada). Scrolls from the paraffin block and pooled frozen tissues were tested with primers for Marek's disease virus<sup>1</sup> and reverse transcriptase PCR, based on work by Islam et al<sup>2,3</sup> in 2004 and 2006 and Baigent et al<sup>4</sup> in 2006. Results were positive.

### Discussion

Marek's disease, caused by oncogenic strains of gallid herpesvirus 2 (serotype 1), an  $\alpha$ -herpesvirus, is an economically important disease of chickens that, in the commercial sector, is typically controlled by vaccination.<sup>4</sup> The virus initially replicates in lymphocytes, inducing destruction of lymphoid tissues.<sup>5</sup> As with  $\gamma$ -herpesviruses, the virus then goes latent in T lymphocytes, with limited antigen expression.<sup>5</sup> This allows the virus to be carried to various organs in the body, including peripheral nerves, skin, and feather follicles. Infected lymphocytes in the nerves and viscera transform and proliferate, resulting in lymphoid tumors.<sup>5</sup> The principle nerves affected by Marek's disease virus include the ischiadic nerve, vagus nerve, and the celiac and brachial plexuses.<sup>5</sup> Viral replication continues in the skin and feather follicles, and shedding of the virus via skin and feather debris acts as the major source of viral exposure to other individuals within the flock.<sup>6</sup> Given the rapid evolution of the virus, along with limited variation in existing vaccines, unexpected outbreaks continue to occur and Marek's disease remains a challenge to the poultry industry.<sup>5</sup> Marek's disease is now regarded as a contagious neoplastic disease of worldwide distribution.<sup>7</sup> Improved biosecurity and genetic resistance are key additions to the existing vaccination programs.<sup>5</sup> Because of variable vaccination regimens, as well as lower biosecurity and, in some



**Figure 5.** Histopathologic images of (A) skeletal muscle and (C) nerve from the left thigh of the chicken described in Figure 1 with Marek's disease and (B and D) an age-matched control chicken. In (A) the affected chicken, there is increased perimysial fibrous connective tissue (asterisks) and separation of, and diffuse variation in, myofiber size, including small, angular fibers and multiple swollen hypereosinophilic myofibers (arrows). Within the left ischiadic nerve, increased numbers of lymphocytes and plasma cells (arrowheads) are present. Occasional dilated myelin sheaths are also present (arrows) (hematoxylin and eosin stain; bar = 100  $\mu\text{m}$  [A and B] or 20  $\mu\text{m}$  [C and D]).

cases, lower hygiene in backyard poultry flocks, infection with Marek's disease virus is common.

Clinically, the classical disease manifestation of Marek's disease is polyneuritis and paralysis, which carries a grave prognosis because there is no effective treatment.<sup>5,7</sup> The polyneuritis associated with Marek's disease is characterized by pathologic changes in the Schwann cells secondary to lymphoid proliferation in the nervous tissues, similar to the histopathologic results in this case.<sup>8</sup> The subsequent demyelination is thought to have a major role in inducing clinical paralysis.<sup>8</sup> Electromyography has been used to assess traumatic brachial plexus injury in red-tailed hawks (*Buteo jamaicensis*) and to monitor organophosphate-induced delayed neuropathy in hens.<sup>9,10</sup>

The neurologic examination localized the lesion to the nerves or muscles of the left pelvic limb. The dramatic rapid muscle atrophy seen in the left pelvic limb was suspicious for denervation. Further investigation was pursued using electromyography. An EMG records the electrical activity of a muscle stimulated by the insertion of an electrode into the muscle.<sup>10</sup> Normal resting muscle is generally electrically silent during inhalational anesthesia.<sup>11</sup> Upon needle placement, mechanical damage to myofibers induces a brief burst of electrical activity.<sup>11</sup> This is termed insertional activity and should not last more than a few hundred milliseconds.<sup>11</sup> Prolonged insertional activity, as was seen in this patient, is a nonspecific indication of injury to the motor unit and does not distinguish between neuropathy and myopathy.<sup>10</sup> In the case described

here, in addition to prolonged insertional activity, the EMG revealed spontaneous fibrillation potentials and runs of positive sharp waves. Spontaneous fibrillation potentials are aberrant action potentials of hypersensitive myofibers seen because of instability of the muscle membrane in abnormal or injured tissue and can be seen in denervation, inflammation, or other myopathies.<sup>10,11</sup> Positive sharp waves are spontaneous electrical potentials with an initial positive (downward) spike, followed by a shorter, slow, negative (upward) potential in a characteristic “saw-tooth” appearance. Positive sharp waves reflect the same underlying pathology as fibrillation potentials.<sup>10,11</sup> In this patient, the multiple, reproducible runs of fibrillation potentials with interspersed positive sharp waves seen in various parts of the abnormal muscles were suggestive of lower motor neuron disease.<sup>10,11</sup> The pattern of affected muscles supported the neurolocalization of a peripheral neuropathy of the tibial and common peroneal branches of the ischiadic (sciatic) nerve as well as part of the obturator nerve.

To our knowledge, this is the first published description of the application of electromyography for the antemortem diagnosis of Marek’s disease in a chicken, which was confirmed by histologic findings and PCR testing. Marek’s disease in chickens is classically diagnosed on postmortem examinations of dead or sacrificed birds as part of a population medicine approach, despite the possibility of antemortem PCR testing.<sup>1</sup> Electromyography is an alternative tool to aid in the antemortem recognition of polyneuritis, often associated with Marek’s disease. Further research investigating the use of EMG, as well as nerve conduction velocity studies in chickens, is warranted to fully characterize the nature of the abnormalities associated with peripheral lymphoid neuropathy induced by Marek’s disease.

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