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MASTICATORY MUSCLE MYOSITIS IN A GRAY WOLF (*CANIS LUPUS*)

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Abstract: A 10-yr-old male, neutered gray wolf (*Canis lupus*) was presented for atrophy of the temporalis and masseter muscles. Clinical signs and magnetic resonance imaging were consistent with a myopathy. Positive serology for antibody titers directed against Type 2M myofibers, and the observation of a mixed mononuclear inflammatory cell infiltrate along with eosinophils and neutrophils within the temporalis muscle, were diagnostic for masticatory muscle myositis. Importantly, protozoal myositis was excluded based on other clinicopathologic data. The case highlights the potential for immune-mediated polymyositis in canids other than the domesticated dog (*Canis lupus familaris*). Additionally, awareness of a diet in which raw meat is used should prompt a thorough investigation for an underlying infectious myositis in the gray wolf.

Key words: *Canis lupus*, gray wolf, masticatory myositis, type 2M myofibers.

BRIEF COMMUNICATION

A 10-yr-old male, neutered gray wolf (*Canis lupus*) presented for atrophy of the temporalis and masseter muscles which had developed over several weeks. The animal was able to eat and drink normally. There was no weight loss or change from normal activity level. For the prior 8 yr, the animal had lived at a privately owned facility in New Jersey, USA, where it was housed outdoors in a fenced-in enclosure along with other wolves; the animal was never allowed outside unsupervised. Vaccinations for canine distemper virus and rabies were up to date per the owner. Diet consisted of a dry kibble (Blue Buffalo Wilderness; Blue Buffalo Co., Wilton, Connecticut 06897, USA), combined with raw meats including chicken legs and thighs sold for human consumption and limb parts of white-tailed deer (WTD; *Odocoileus virginianus*) obtained from local hunters. No other animals were affected.

With the exception of pronounced atrophy of the temporalis and masseter muscles, physical examination was normal. Neurologically, the animal had a normal mental state, gait, postural reactions, and spinal reflexes. Cranial nerve examination was normal, including normal sensation in the autonomous zone for the maxillary, ophthalmic, and mandibular branches of the trigeminal nerve bilaterally. There was no resistance or pain noted when the mouth was opened or closed or with palpation of the muscles. The neuroanatomic diagnosis was consistent with a disease process involving the muscles of mastication (MM).

The complete blood count, serum chemistry profile, and creatinine kinase level[11] were normal. Serum concentration of thyroid stimulating hormone, total thyroxine and free thyroxine, and urinalysis were normal.[14] Thoracic radiographs were normal. Prior to general anesthesia for magnetic resonance imaging (MRI) of the head, hydromorphone (2 mg/ml injectable solution, Hydromorphone HCl, Hospira, Inc., Lake Forest, Illinois 60045, USA; 0.05 mg/kg i.v.) was administered. General anesthesia was induced with propofol (Abbott Laboratories, North Chicago, Illinois 60064, USA; 4–6 mg/kg i.v.). In the event of insufficient anesthetic depth, a propofol bolus was given. The MRI of the head was performed using a 1.5 T MRI unit (GE Signa Excite, GE Healthcare, Milwaukee, Wisconsin 53201, USA). Pulse sequences acquired included

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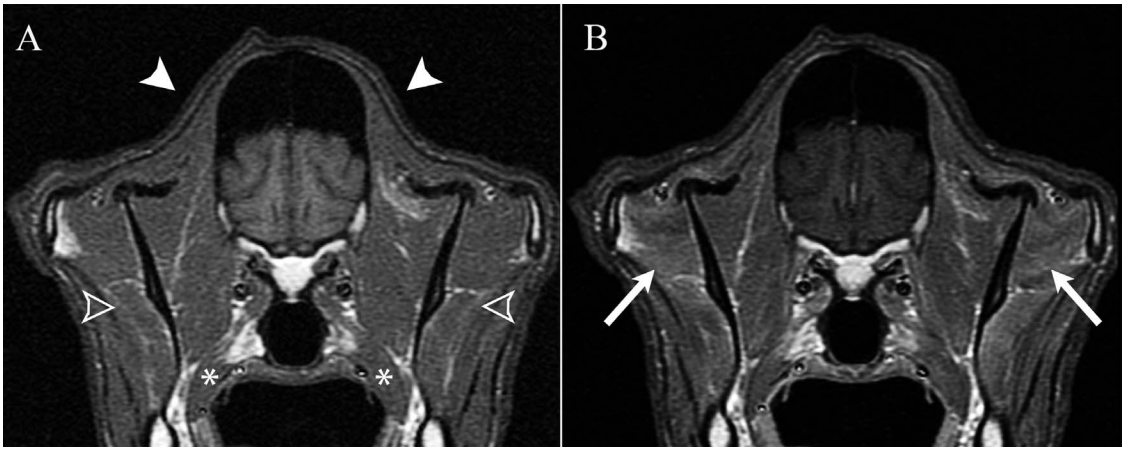

Figure 1. Masticatory myositis. **A.** Transverse plane, T1-weighted MRI sequence of the head of a gray wolf at the level of the ramus of the mandible. There is pronounced atrophy of the temporalis muscles (arrowheads) along with atrophy of the masseter (open arrowheads) and pterygoid muscles (asterisks). **B.** Following intravenous contrast administration, there is ill-defined, heterogeneous contrast enhancement of the muscles of mastication, which is most conspicuous in the masseter muscles (arrows).

T2-weighted (T2W), proton density, T2W fluid attenuated inversion recovery (T2W-FLAIR), and T1-weighted (T1W) sequences. Additional T1W sequences were acquired following intravenous administration of gadodiamide (0.1 mmol/kg Omniscan, GE Healthcare, Princeton, New Jersey 08540, USA). The MRI disclosed severe atrophy of the MM: temporalis and masseter muscles with lesser atrophy of the pterygoids and the diagastrus muscles bilaterally. The MM were characterized by multifocal, ill-defined regions of hyperintensity on T2W and T2W-FLAIR images. The MM were isointense on T1W images and displayed heterogeneous contrast enhancement. The MRI was consistent with a disease process involving the MM, such as an inflammatory myopathy (Fig. 1).

Following MRI, an incisional biopsy[6] of the temporalis muscle was made for histopathologic examination of the MM. Using a serologic assay validated for the domestic dog, the titer of antibodies directed against the Type 2M myofiber was 1 : 4,000 (normal = <1 : 100), consistent with MM myositis. Other serologic testing results included *Toxoplasma gondii* IgM = 1 : 512 and IgG = 0; *Neospora caninum* >1 : 40; canine distemper virus (CDV) IgM = <1 : 10 and IgG = 1 : 80; and *Cryptococcus neoformans* was negative. Given the serum concentration for Type 2M autoantibodies, the serology for infectious agents was interpreted as exposure to *T. gondii* and *N. caninum* and related to vaccination for CDV, respectively. In support of this, repeated titers 30 days later were similar (*T. gondii* IgM = 1 : 256 and IgG = 0; *N. caninum* >1 : 40). Recovery from anesthesia was uneventful.

Microscopically, the temporalis muscle contained perimysial and endomysial inflammatory infiltrate composed of mononuclear cells (lymphocytes, macrophages, and plasma cells) along with lesser numbers of eosinophils and neutrophils (Fig. 2). The inflammation varied from mild to severe, wherein the inflammation entirely replaced the myofibers. The inflammation was concentrated around vessels and around individual myofibers (Fig. 2, inset). Myofibers varied in size. Myofiber necrosis, as evidenced by an eosinophilic cytoplasm and loss of striation, and regeneration, as evidenced by basophilic cytoplasm and centralized nuclei, were present. Phagocytosis of necrotic myofibers was observed. There was mild perimysial fibrosis. Immunohistochemistry for *Sarcocystis neurona* was negative.

Based on the clinical signs, the presence of antibodies directed against Type 2M myofibers, and on the MRI and microscopic findings, a diagnosis of MM myositis was made. The animal was treated with prednisone at 0.4 mg/kg orally twice daily for 30 days, at which point the dosage was reduced by 25% every 30 days (Prednisone, West-Ward Pharmaceutical Corp., Eatontown, New Jersey 07724, USA). After 4 mo, repeated serology for antibodies directed against the Type 2M myofiber was 1 : 100 (normal = <1 : 100).

In the domestic dog (*C. l. familiaris*), inflammatory myopathies are categorized as infectious,

paraneoplastic, or immune-mediated.[5] Infectious agents associated with myositis include protozoa, bacteria, spirochetes, and fungi. Paraneoplastic myositis is most-commonly associated with lymphoma and thymoma.[5] The term polymyositis is reserved for inflammatory myopathies with a known or presumed immune-mediated basis.[5] Polymyositis may be generalized or focal; focal polymyositises include MM myositis and extraocular myositis.[5] Given its name, MM myositis affects the muscles of mastication: temporalis, masseter, ptyerygoids, and rostral portion of the digastricus. Other muscles are spared. Two clinical presentations are observed, including an acute form characterized by marked swelling of the MM, most visible in the temporalis and masseter muscles, and a chronic form characterized by atrophy of the MM. In addition, there may be pain on opening the mouth or an inability to open the mouth.[8,13]

The MMs are derived from the first branchial arch (visceral arch) mesoderm. As a consequence, the MM contain a unique myosin isoform which is designated Type 2M. In dogs with MM myositis, autoantibodies directed against the unique myosin heavy chain, myosin light chain, and masticatory myosin binding protein-C are present in serum.[15] The detection of autoantibodies in sera forms the basis of the diagnosis, providing an 82% diagnostic sensitivity and 100% specificity.[13] Autoantibodies directed against the Type 2M myofiber are not present in dogs with generalized polymyositis, even when there is involvement of the MM.[13] Therefore, the development of autoantibodies directed against the Type 2M myofibers is not secondary to myofiber damage.

Microscopically, affected muscles contain myofiber necrosis and phagocytosis, an inflammatory cell infiltrate composed of mononuclear cells and occasionally eosinophils, and fibrosis.[8,13] The mononuclear infiltrate is characterized by a predominance of B-cells, dendritic cells, and macrophages out-numbering T cells, more CD4+ T cells than CD8+ T cells, and T cells with TCR$\gamma\delta$, suggesting a different pathophysiology than with generalized polymyositis.[12]

While not a definitive diagnostic test, MRI provides clinical information and assists in selecting an appropriate biopsy site.[2] Definitive diagnosis is established through finding serum autoantibodies directed against Type 2M myofibers, histology of affected MM, and the exclusion of other etiologies. In lieu of serum autoantibodies directed against Type 2M myofibers, the demonstration of IgG bound to affected muscle using staphylococcal protein A conjugated to horseradish peroxidase suggests an underlying immune-mediated myositis.

To the authors' knowledge, this is the first report of MM myositis in the gray wolf. Masticatory muscle myositis appears unique to the dog with reported cases only involving domestic dogs. While histologically similar, MM myositis occurs in mink; however, autoantibodies directed against the type 2M myofibers or in situ identification of antibodies on muscle specimens were not found.[10]

As in affected domesticated dogs, the presence of Type 2M autoantibodies in serum, and microscopic identification of a mononuclear inflammation in the MM, established the diagnosis in this case. Although we were unable to pair the serum of this affected wolf with serum of an unaffected gray wolf, we are confident that the commercially available assay for Type 2M autoantibodies in domestic dogs can accurately detect the presence of these same autoantibodies in the gray wolf, given the close phenotypic and genotypic similarity of the wolf with the domesticated dog.[9]

In affected dogs, treatment consists of immunosuppression using corticosteroids. This highlights the need to exclude an infectious etiology. Relevant in the present case, the diet included raw meats consisting of chicken and WTD. Numerous pathogens, including protozoal agents, have been identified in commercially available and home-prepared raw meat-based diets (RMBDs).[7] Of reported pathogens in RMBDs, protozoal organisms have the potential to cause myositis. The WTD represents an important sylvatic reservoir for the protozoal agents *Toxoplasma gondii*, *Neospora caninum*, and *Sarcocystis* spp. In the United States, serologic surveys of WTD report the prevalence of antibodies against *T. gondii* that range from 28.7–64%,[3] the prevalence of *N. caninum* antibodies that range up to 50%,[14] and the prevalence of *Sarcocystis* spp. that range up to approximately 40% of all animals.[1] Consequently, it was imperative to exclude these protozoa from consideration.[4] Ultimately, immunohistochemistry and repeated serology ruled out protozoal infection.

The present case documents MM myositis in a gray wolf. Signs parallel those observed in the domestic dog. Although a single case, the results suggest the Type 2M antibody serology can be used in gray wolves. This case provides clinicians working with nondomestic canids an important differential diagnosis for animals with lesions involving the MM. As important as recognizing the existence of MM myositis in the gray wolf is

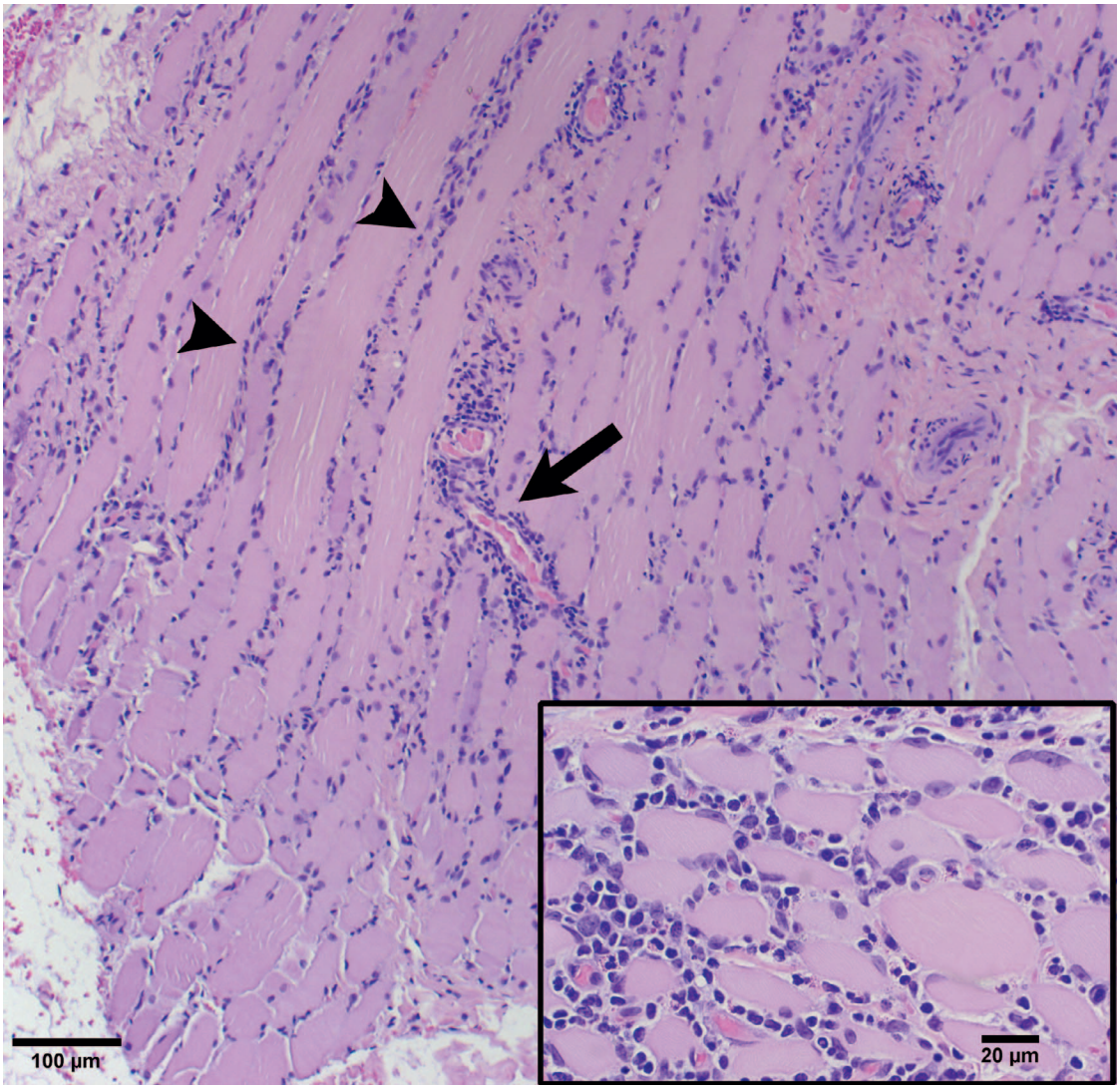

Figure 2. Masticatory myositis. Microscopically there is endomysial (arrowheads) and perivascular (arrow) inflammation in the temporalis muscle. Hematoxylin and eosin (H&E), ×10. Bar = 100 µm. Inset: The endomysial inflammatory infiltrate is composed primarily of a mixed mononuclear cell population including lymphocytes, macrophages, and plasma cells. Occasional eosinophils are present. Myofibers display variation in size. H&E, ×40. Bar = 20 µm.

that a consideration of MM myositis should also include a thorough diagnostic workup that excludes infectious myositis.

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