CONTENTS

First Report of *Hepatozoon* spp.[1] In a Dog at the Paso del Norte Region, US-Mexico

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Abstract. Canine hepatozoonosis is a tick-borne disease caused by apicomplexan hemoparasites of the genus *Hepatozoon* spp. (Apicomplexa: Hepatozoidae). We report a clinical case of hepatozoonosis in a domestic Siberian husky dog, *Canis lupus familiaris* (Linnaeus). The dog was a male of 2 years remitted to the University Veterinary Hospital because it appeared lame in the hind limbs. During clinical evaluation it presented ataxia, loss of proprioception in both hind limbs, hyperreflexia of the right hind limb, and hyporeflexia of the left hind limb. Intervertebral disc disease was ruled out with radiographic and magnetic resonance imaging. Serological tests for infectious diseases such as ehrlichiosis, anaplasmosis (SNAP 4Dx Plus® test, IDEXX Laboratories), and leptospirosis (SNAP® Lepto Test, IDEXX Laboratories) were negative. Four biopsy samples of the biceps femoris muscle were taken. Histopathological diagnosis reported "onion skin" cysts 200 to 300 µm, suggestive of *Hepatozoon americanum*. Clinical confirmation of *Hepatozoon* spp. at the border of Mexico with Texas and New Mexico could impact differential diagnosis of neuromuscular disease in the area, especially in dogs where the causes of muscular pain and weakness and lameness are non-diagnostic.

Canine hepatozoonosis is a disease transmitted by ticks infected with protozoa of the Hepatozoidae family. Unlike other tick-borne diseases, infection does not occur through blood, but when a dog, *Canis lupus familiaris* (Linnaeus), swallows a tick. To date, dogs can be infected by two tick-borne *Hepatozoon* spp.: *H. canis* and *H. americanum*. Although phylogenetically related, the two species differ in a variety of aspects that include clinical signs, life cycles, and the tick that acts as the definitive host and transmitter. In Europe, Africa, and Asia, *H. canis* is the prevalent species that causes infection. In contrast, dogs in the southern USA have been infected with *H. americanum*, while in Central and South America both *Hepatozoon* species occur.

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The brown dog tick, *Riphicephalus sanguineus* (Latreille), is the definitive host and responsible for transmission of *H. canis*. The Gulf Coast tick, *Amblyomma maculatum* (Koch), is the transmitting tick and definitive host of *H. americanum* (Baneth et al. 2003, Ewing and Panciera 2003).

Severity of disease caused by the two species of hepatozoon also differs. For example, dogs infected with *H. canis* can present various ranges of infections, oscillating from subclinical states in dogs with low parasitemia to severe manifestation of the disease with fever, lethargy, anemia, and emaciation that can be life-threatening in pronounced parasitemia (Thakur et al. 2018, Sahu et al. 2019, Medkour et al. 2020). Conversely, dogs infected with *H. americanum* frequently have more severe onset of the disease, characterized by fever, myalgia, myasthenia, and depression. Dogs regularly have gait abnormalities and muscle wasting, with atrophy of head muscles. Also, copious mucopurulent ocular discharge is common in most dogs. Affected animals may have normal appetite, but often refuse to move to food and water, probably because of intense pain associated with periosteal bone proliferation and myositis (Ewing and Panciera 2003).

A 2-year-old Siberian husky dog was remitted for clinical review to the University Veterinary Hospital of the Universidad Autónoma de Ciudad Juárez. The dog had lameness in both hind limbs. In the medical history, the owner stated the dog traveled to a forest in New Mexico 2 months before and was infested by ticks. The dog was successfully treated in another veterinary hospital where the tick species were not identified. During the clinical exam, the dog presented proprioception failure in both hind limbs, ataxia, hyperreflexia of the right hind limb, and hyporeflexia of the left hind limb, accompanied by marked muscle weakness.

In routine analytical tests, all parameters were in range, except a slight leukocytosis of 15.37 x 10^3 cells/ml (range 4.36-14.8 x10^3 cells/ml) with monocytosis. Radiographic images did not show any obvious abnormalities. Plain radiographs, myelography, and magnetic resonance were done. It was ruled out that the dog could present intervertebral disc disease or fibrocartilaginous embolism. Serological tests were done for ehrlichiosis, anaplasmosis, Lyme disease, and leptospirosis, all with negative results. Supportive treatment was administered with carprofen 4.4 mg/kg orally, once daily; tramadol 3 mg/kg orally, three times a day and omeprazole 0.7 mg/kg orally, once a day. During subsequent examination, the dog always showed weakness and muscle pain. However, the dog sometimes alternated from hyperreflexia and hiporreflexia. Therefore, it was thought the injury was extramedullary.

As a final diagnostic test, the dog was taken to the surgery area to obtain four biopsies of the biceps femoris muscle. The owner decided to perform the biopsy on Day 90 after the first review. Results of the pathophysiological study showed an eosinophilic structure 200 to 300 µm in longitudinal section that had the appearance of "onion skin" (Fig. 1), which suggested a protozoan cyst compatible with *H. americanum*.

With the results, we started treatment aimed at *H. americanum* infection: trimethoprim/sulfadiazine 15 mg/kg, orally, twice a day; clindamycin 10 mg/kg, orally, thrice a day; pyrimethamine 0.25 mg/kg, orally, once a day; decoquinate 15 mg/kg, once a day; and prednisone 0.5 mg/kg, orally, once a day. The dog showed marked improvement during the first 20 days of treatment, improving his mood, walking, muscle tone, and mass. However, 2 months after treatment, the dog relapsed. The dog showed leukocytosis of 30 x10^3 cells/ml, as well as severe thrombocytopenia of 40,000 platelets/ml. Also, the liver enzymes AST, ALT, FAS, GGT, and total bilirubin

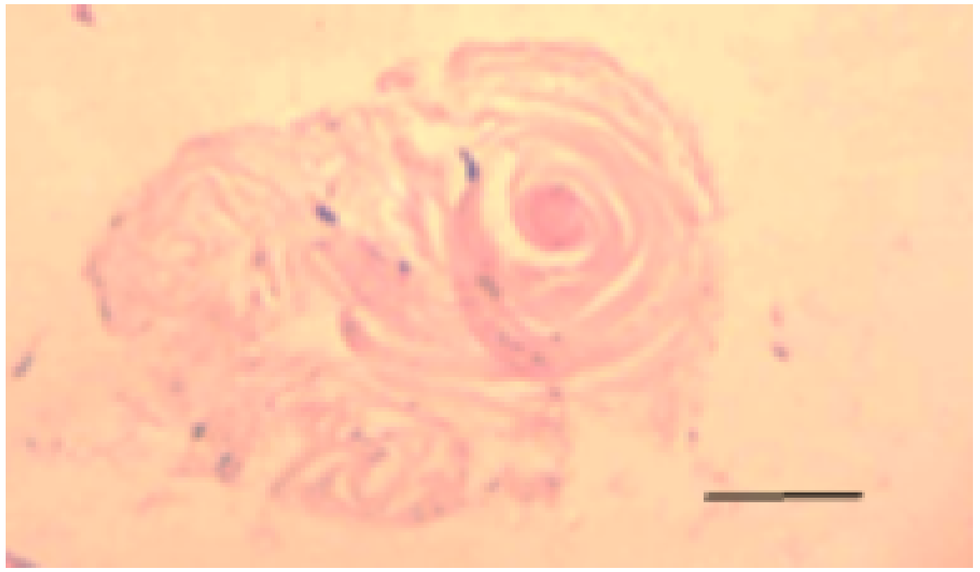

Fig. 1. Muscle biopsy of the dog where characteristic cyst "onion skin" was observed. 400x magnification, 50 µm scale bar.

were elevated. After 45 days of treatment, the condition of the dog continued to worsen and became unresponsive to treatment. At which time, the owner decided to euthanize the dog.

Canine hepatozoonosis is a tick-borne disease. The causative agent in infected dogs from Europe, Asia, Africa, and South America was *H. canis* whose definitive host is the brown dog tick, *R. sanguineus* (Baneth et al. 2003). *H. americanum* is habitually recognized as an emerging disease in endemic areas of the USA, whose definitive host and vector is *A. maculatum*. At first, the geographical range in the USA was limited to one region of the southeastern states, which is the exclusive habitat of *A. maculatum*. However, recent evidence supports the possibility that the range is expanding as far north as Kansas and Kentucky (Potter and Macintire 2010). Currently, there are case reports of dogs infected with *H. canis* in the United States, in Alabama, Georgia, Mississippi, Louisiana, and Virginia (Li et al. 2008). In Mexico, are isolated reports of canine hepatozoonosis. One occurred at Tamaulipas, a border state with eastern Texas, where it was believed *H. americanum* was the causative agent of the disease (Carvajal et al. 2012). In other research in the southern state of Tabasco, PCR showed19 dogs positive for *H. canis* (Jarquín-Díaz et al. 2016).

Limitation of the work is that diagnosis by molecular biology was not established. This might have differentiated between the two species of *Hepatozoon* spp. that infect dogs. However, in the clinical case, infection by *H. americanum* was strongly suspected. The clinical signs (weakness and muscle pain with lameness) were less severe than *H. canis* described as causing less severe disease (Baneth et al. 2003, Ewing and Panciera 2003, Baneth 2011, Carvajal et al. 2012). Further, confirmation of the parasitic cyst in muscle biopsies were very diagnostic of *H. americanum* (Ewing et al. 2003). Histopathology revealed pyogranulomatous

myositis and large round to oval cysts (250-500-micrometer diameter) known as "onion skin" (Baneth et al. 2003, Baneth 2011) confirmed in the histopathological report of the case. Although *H. canis* also can be diagnosed microscopically, differences were: 1) it is more common to detect intracellular gamonts within neutrophils in stained blood-smears, and 2) mature forms of *H. canis* are rarely found in the muscle which has characteristic morphology formed by elongated merozoites organized in a circle around a clear central core, developing a unique shape of a "wheel spoke" (Ewing and Panciera 2003, Baneth 2011).

It was difficult to establish how the dog was infected. The tick *A. maculatum* only is reported in a small geographical area, only the southeastern region in Texas near the Gulf of Mexico (Potter and MacIntire 2010, Sonenshine 2018). The dog had a history of being infested by a tick in New Mexico, a state without reported *A. maculatum*. However, the tick already has been described farther west, in Arizona (Allerdice et al. 2017, Sonenshine 2018). Another possible infection pathway was that the dog had hunted a prey harboring infected *Amblyomma* spp. or containing cystozoites of *Heptazoon* spp. in their tissues, ever since recent studies demonstrated that predation is an important transmission source of *H. americanum* (Johnson et al. 2009, Carvajal et al. 2012). Another species of *Amblyomma* spp. might be a possible transmitter of the disease. For example, *A. cajennense* and *A. imitator* were described in Mexico in a dog with hepatozoonosis, but transmission was not demonstrated.

Although the dog first responded adequately to treatment, long-term treatment was not successful. The dog presented severe leukocytosis with thrombocytopenia, in addition to elevated liver enzymes (AST, ALT, ALP, and GGT), as well as bilirubin. If the protocol was not strictly used, relapse of disease likely will occur (Ewing and Panciera 2003) and the cystic form in muscles is not eliminated from dogs (Johnson et al. 2008). Severe thrombocytopenia is not common in dogs with *H. americanum*, so it could be possible that in the present case the animal had co-infection with another hemoparasite. Changes in blood biochemistry also can be explained by co-infection of hemoparasites or that are secondary to drug-induced hepatopathy. To our knowledge, this is the first report of canine hepatozoonosis in the Paso del Norte metropolitan area, comprised of the urban areas of El Paso, TX and Las Cruces, NM in the USA and Juarez, Chihuahua in Mexico.

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