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Source: Journal of Wildlife Diseases, 35(1) : 86-89

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-35.1.86>

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Naturally Occurring Hepatozoonosis in Coyotes from Oklahoma

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ABSTRACT: Nine of 16 free-ranging coyotes (*Canis latrans*) from central Oklahoma (USA) had naturally acquired infections of *Hepatozoon americanum*. Infections were confirmed by recognition of tissue stages closely resembling *H. americanum* in skeletal and cardiac muscle. At the time coyotes were collected they were infested with a variety of ticks, including adult Gulf Coast ticks (*Amblyomma maculatum*). We propose that the high prevalence of *H. americanum* in this small sample of free-ranging coyotes and the ability of these same animals to harbor adult populations of *A. maculatum* is an important component of the epizootiology of canine hepatozoonosis in North America.

Key words: *Amblyomma maculatum*, *Canis latrans*, coyotes, Gulf Coast tick, *Hepatozoon americanum*, survey.

Hepatozoon americanum (Apicomplexa: Adeleina) recently has been described as a new species and implicated as the causative agent of canine hepatozoonosis in the United States (Vincent-Johnson et al., 1997). This organism is differentiated from *Hepatozoon canis*, an Old World species, on the basis of clinical signs produced in infected dogs, histopathological lesions, serological findings, and gamont size and morphology (Vincent-Johnson et al., 1997). The geographic distribution of the parasite in the USA, based on reported cases in domestic dogs, is Alabama, Georgia, Louisiana, Mississippi, Texas, and Oklahoma (Craig et al., 1978; Gaunt et al., 1983; Barton et al., 1985; Gosset et al., 1985; Macintire et al., 1997; Vincent-Johnson et al., 1997; Panciera et al., 1997). However, it is speculated that the distribution may include most of the southeastern USA (Vincent-Johnson et al., 1997).

The inconsistency and/or inability of several tick species, including the *H. canis* vector, *Rhipicephalus sanguineus*, to trans-

mit *H. americanum* has been reported by several authors (Nordgren and Craig, 1984; Vincent-Johnson, 1997; Mathew et al., 1998). This fact and the recent discovery that the Gulf Coast tick (*Amblyomma maculatum*) can serve as an experimental vector for *H. americanum* (Mathew et al., 1998) raises many questions regarding the epizootiology of this infection. It brings into consideration the numerous hosts of *A. maculatum* as potential participants in the epizootiology of this infection in domestic dogs in North America.

Amblyomma maculatum has only recently become recognized as a major pest in areas distant from the Gulf Coast (USA). Reports from Oklahoma (Semtner and Hair, 1973) first indicated a considerable annoyance of cattle in the eastern portion of the state with the northeastern region most significantly impacted. Additional reports from Kansas and Kentucky (USA) also identified cattle as a preferred host for adult *A. maculatum* (Wiedl, 1981; Snoddy, 1984). While cattle appear to be the most common host for adult ticks, coyotes, domestic dogs, and jackrabbits (*Lepus californicus*) also harbor this stage. Immature stages were found on a wide range of birds and mammals with both larval and nymphal *A. maculatum* infesting coyotes (Semtner and Hair, 1973).

Samuel and Trainer (1970), Kellogg et al. (1971), and Durden et al. (1991) reported white-tailed deer (*Odocoileus virginianus*) to be an infrequent host for adult *A. maculatum* in several locations in the southeastern United States. Mercer et al. (1988) reported *A. maculatum* from bobcats (*Lynx rufus*) and ocelots (*Felis pardalis*) in Texas.

The most recent data available estimates coyote populations in eastern Oklahoma at 0.5 to 1.0 coyotes/mile² (0.2 to 0.4 coyotes/k²) of suitable agricultural land. Total population estimates, made annually for eastern Oklahoma, have remained steady or increased yearly since 1981, presently ranging between 10,000 and 20,000 animals (Anonymous, 1996).

The documentation of naturally occurring cases of hepatozoonosis in free-ranging coyotes has been limited to the immediate Gulf Coast region of Texas. At the time of reporting, the Gulf Coast was the only geographic area in the United States where hepatozoonosis was known to be enzootic in carnivores (Davis et al., 1978; Mercer et al., 1988).

Recognition of canine hepatozoonosis in domestic dogs in Oklahoma (Panciera et al., 1997, 1998) prompted speculation that a wild canid may be involved in both maintenance and distribution of this parasite. To evaluate this possibility, 16 adult coyotes ranging from 3- to 8-yr-old were obtained from three locations in central and northcentral Oklahoma (35°50'N to 36°50'N, 97°00'W to 98°00'W) during April 1998. Animals were shot as a part of predator damage management (United States Department of Agriculture, Animal Damage Control, Washington D.C., USA). Immediately upon death, blood samples were taken, blood smears were prepared and animals were placed in individual plastic bags and transported to the Oklahoma Animal Disease Diagnostic Laboratory (Stillwater, Oklahoma, USA). Serum was harvested, blood films were fixed in 100% methanol and stained with Diff-Quick® (Dade Diagnostics, Inc., Aguada, Puerto Rico) and ticks were collected and identified. No effort was made to collect the total tick population on the coyotes, but the presence and number of individual species removed was recorded. Representative specimens of *H. americanum* (Accession number 088005) and *A. maculatum* (Accession number 088004) were deposited in

the U.S. National Parasite Collection (Beltsville, Maryland, USA).

Gross pathologic findings were generally unremarkable with most animals appearing to be in good nutritional condition. A single subcutaneous abscess in the cervical region of one animal was observed and mild generalized lymph node enlargement was evident in several animals. At necropsy, specimens of cardiac and skeletal muscle, spleen, lymph node and liver were collected from each coyote and fixed in 10% buffered formalin. Multiple sections of skeletal and cardiac muscle and representative sections of other tissues were embedded in paraffin, sectioned at 5 µm, stained with hematoxylin-eosin, and examined by light microscopy.

Microscopic lesions compatible with *H. americanum* were observed in striated muscle in nine of 16 coyotes. Parasite states observed included round to ovoid, lamellated "onion skin" cysts and granulomas containing zoites. All parasitic lesions observed except one were in skeletal muscle; the exception was a "cyst" in cardiac muscle. The density of infection varied with 12 lesions observed in muscle specimens of one animal, nine lesions observed in muscle specimens from another, and one to three lesions in muscle of seven of the 16 coyotes. The cysts were generally ovoid; the largest was 110 × 400 µm, the smallest 80 × 170 µm and were composed of pale staining or mildly lamellated material. Although most cysts did not contain a demonstrable central cell, the centrally located cells that were apparent were 20 to 25 µm diameter (Fig. 1) with distinct cytoplasmic borders, an eosinophilic granular cytoplasm and a 6 µm eccentric nucleus. Two lesions were composed of discrete granulomas (Fig. 2). One was 120 µm × 210 µm, ovoid, and of relatively low cellularity consisting predominantly of neutrophils in a connective tissue stroma. A few of the neutrophils had crescentic nuclei displaced by a 4 µm zoite within the cytoplasm. The zoites (not shown) were spherical, slightly basophilic and refractile.



FIGURE 1. "Onion-skin cyst" of *Hepatozoon americanum* in muscle of a coyote from Oklahoma. The plane of the histologic section has apparently missed the cell nucleus in the centrally located host cell. The discrete intracellular body (arrow) is believed to be parasite. Note lamellar arrangement of mucopolysaccharide surrounding the host cell. H & E Bar = 50 μ m.



FIGURE 2. Discrete focus of pyogranulomatous inflammation (granuloma) populated by neutrophils, monocytes and sparsely distributed eosinophils in the skeletal muscle of a coyote from Oklahoma infected with *Hepatozoon americanum*. Zoites are not apparent in this section but were seen in others. H & E Bar = 50 μ m.

The other granuloma was 120 μ m \times 450 μ m and moderately cellular with neutrophils and a few macrophages. Zoites were not visible in this lesion.

Although *H. americanum* lesions present in the coyote are morphologically similar to those seen in domestic dogs, the inflammatory cell component of the two granulomas examined in these coyotes was somewhat less cellular than that seen in comparable lesions in the dog. Neutrophils are major participants in the early stages of granuloma formation in the dog but are largely replaced by mononuclear macrophages in later stages of the inflammatory process (Pancier et al., 1998).

Gamonts were not observed in any of the blood smears examined. At the time of necropsy, all 16 coyotes were infested with adult *Dermacentor variabilis*, *Amblyomma*

americanum, and *A. maculatum*. Additionally, three of the coyotes had adult *Ixodes scapularis* and seven had nymphal *A. americanum*. The maximum number of ticks collected from individual coyotes ranged from 26 to 80.

These findings confirm the coyote as a natural intermediate host for *H. americanum* in Oklahoma and, based on recent experimental studies of transmission (Mathew et al., 1998), *A. maculatum* is further implicated as a potential natural epidemiologic bridge between wild and domestic canids. As has been proposed by others (Mercer et al., 1988; Vincent-Johnson, 1997), a susceptible wildlife reservoir that is abundant and cosmopolitan in distribution and that could maintain infected tick populations is a more likely natural host for this organism than is the domestic dog. This is believed to be the first report of *H.*

americanum in free-ranging coyotes in any location away from the immediate coastal region of Texas.

We thank J. Lilley and D. Alley for their assistance in obtaining coyotes. This research was supported in part by Animal Health Grant no. 1433 and project 3714 of the Agricultural Experiment Station, Oklahoma State University.

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Received for publication 9 July 1998.