Members of the genus *Hepatozoon* are unique, heteroxenous hemogregarines in their oral routes of infection to vertebrate intermediate hosts and polysporocystic oocyst formation in invertebrate definitive hosts.\(^1\) Conventionally, the accepted primary route of transmission of *Hepatozoon* spp to vertebrate intermediate hosts is by ingestion of hematophagous, arthropod, definitive hosts containing sporulated oocysts.\(^1,2\)

Currently, 2 *Hepatozoon* spp are recognized parasites of domestic dogs (*Canis familiaris*) in the United States: *H canis* and *H americanum*.\(^3–5\) *Hepatozoon canis*, first observed in the blood of domestic dogs in India in 1905, is now documented in many areas of the world including Africa, Southeast Asia, the Middle East, southern Europe, and South America.\(^2,5,6–8\) However, *H canis* was not definitively identified in canids in the United States until 2008, despite the presence of its accepted primary definitive host and tick vector, *Rhipicephalus sanguineus*, throughout North America.\(^3,5,9,10\)

The first natural canine *Hepatozoon* sp infection in the United States was reported in a coyote (*Canis latrans*) in Texas near the Gulf Coast in 1978.\(^7,11,12\) Reports in domestic dogs in Texas and other states bordering the Gulf Coast soon followed, but the etiologic agent, *Hepatozoon americanum*, was not recognized as distinct from *H canis* until 1997.\(^7,8,13\) The accepted primary definitive host and tick vector of *H americanum* is *Amblyomma maculatum*, the Gulf Coast tick.\(^14,15\)

*Hepatozoon canis* and *H americanum* differ in numerous aspects including geographic distribution, definitive tick hosts, sites of merogony and resulting clinical syndromes in canine intermediate hosts, treatment approaches, and regions of 18S rRNA gene sequence.\(^6,7,15–20\) This chapter reviews much of what is known about canine hepatozoonosis in both the Old World and New World. Emphasis is given to
more recent research findings that have provided insight into the epizootology of canine hepatozoonosis in North America.

GEOGRAPHIC DISTRIBUTIONS, PREVALENCE ESTIMATIONS, AND POSSIBLE WILDLIFE RESERVOIR HOSTS OF H CANIS AND H AMERICANUM

Prevalence Estimations in Domestic Dogs in the United States and Possible Wildlife Reservoir Hosts

_Hepatozoon canis_ was first discovered by S.P. James in 1905 in the blood of domestic dogs (Canis familiaris) in India.2,17 Since its discovery, this parasite has been reported in dogs in many areas of the world, including Europe, Asia, Africa, and South America.2,5,7 _Hepatozoon canis_ was not thought to be a parasite infecting domestic dogs in the United States since the recognition of _H americanum_. However, in 2008, 2 separate survey studies evaluating genetic data of _Hepatozoon_ spp amplified from domestic dogs in the United States molecularly confirmed the presence of _H canis_.3,4 Allen and colleagues (2008) obtained 2 identical sequences from 2 dogs housed in an animal control shelter in Oklahoma that were 98.8% identical to a sequence previously documented as _H canis_. Li and colleagues (2008) reviewed quantitative PCR results from 614 dogs with clinical signs of hepatozoonosis from all over the United States and discovered _H canis_ infections and coinfections of _H canis_ and _H americanum_ in approximately 5% of the animals evaluated. _Hepatozoon canis_ and _H americanum_ coinfections were documented in Alabama, Georgia, Louisiana, Mississippi, Oklahoma, and Virginia. These molecular studies documented evidence of _H canis_ infections and _H canis_ and _H americanum_ coinfections in domestic dogs in the United States for the first time.3,4 It remains unclear whether _H canis_ infections in North America are autochthonous or are the result of introduction through increased international travel practices.

Although _H canis_ is mainly identified in domestic dogs, this parasite has been reported in jackals, hyenas, and palm civets in other areas of the world; however, the species of _Hepatozoon_ infecting these wild carnivores have not been confirmed.21 Recently, genetic sequences most identical to those documented as _H canis_ were obtained from red foxes (Vulpes vulpes) in Italy22 and Croatia23 and domestic cats (Felis catus) in France,24 Thailand,25 and Brazil.26

_Hepatozoon americanum_ was first reported by Davis and colleagues in a coyote (Canis latrans) in Texas in 1978.11 Over the next 2 decades, _H americanum_ was reported in domestic dogs in several states in North America including Texas, Louisiana, Mississippi, Alabama, Georgia, and Oklahoma.7,13,16,27 Initially, these infections were attributed to a particularly virulent strain of _H canis_. Further research on the North American parasite indicated that it was distinct from _H canis_, and in 1997, it was recognized as the causative agent of American canine hepatozoonosis (ACH).10,13,17,28 Although reports of ACH have traditionally occurred in south-central and southeastern states where the accepted tick definitive host and vector of _H americanum_ is established, genetic data of _H americanum_ infections in clinically presenting dogs in additional states were reported in 2008.4 _Hepatozoon americanum_ infections are now documented in California, Kentucky, Nebraska, North Carolina, Virginia, Vermont, and Washington, in addition to states previously reported.4

The overall prevalence of _H americanum_ in the United States is not well understood. In the largest survey study published, 614 blood samples collected from clinically presenting dogs from 28 states were submitted to the Molecular Diagnostics Laboratory at Auburn University between 2006 and 2008 for PCR confirmation of _H americanum_ infection.4 Approximately 30% of the specimens tested were positive for _H americanum_ DNA, the majority of which were submitted from states in the southeast.4
However, this estimation may be lower than the actual infection prevalence because muscle biopsy, rather than whole blood, is considered ideal for detecting *H. americanum* infection, as parasitemia in ACH patients and biopsy-confirmed, experimentally infected dogs, is often extremely low.\(^{12,29}\)

Survey studies in areas of Oklahoma where ACH is enzootic have revealed that approximately half of evaluated coyotes have muscle stages of a parasite that resemble those seen in domestic dogs infected with *H. americanum*.\(^{9,19,30,31}\) Cross-transmission studies between dogs and coyotes indicated that both hosts were likely infected with the same parasite.\(^{32}\) Based on the prevalence of *H. americanum* in coyotes in enzootic areas, some researchers suspect coyotes are an important reservoir host of the parasite\(^{30,32}\) while others conjecture that both domestic dogs and coyotes are accidentally inserted into a transmission cycle involving *A. maculatum* and another, unidentified reservoir host in nature.\(^{12}\) Coyotes appear to tolerate *H. americanum* infection better than dogs; however, naturally infected coyotes develop pathognomonic muscle and bone lesions, and experimentally infected animals display clinical disease consistent with ACH.\(^{9,19,30–33}\)

**DEFINITIVE TICK HOSTS OF *H. CANIS* AND *H. AMERICANUM***

The primary definitive host and tick vector of *H. canis* was identified by Christophers in 1907 as *Rhipicephalus sanguineus* (Fig. 1), the brown dog tick.\(^{2,17}\) *R. sanguineus* nymphs have been experimentally demonstrated to support *H. canis* oocyst formation after repletion feeding on infected dogs or after percutaneous injection with buffy coat from infected dogs.\(^{2,34}\) Larvae are apparently refractory to infection.\(^{10,15}\) Mature oocysts are found approximately 53 days post-repletion in 66% to 85% of molted adult cohorts infected with *H. canis* as nymphs.\(^{2,34}\) It is not entirely clear where zygote formation and sporogony occur within the tick, or whether these processes take place intracellularly or extracellularly.\(^{2}\) Experiments assessing transovarial transmission of *H. canis* in *R. sanguineus* indicate this route does not occur.\(^{34}\)

*Rhipicephalus sanguineus*, a 3-host tick that preferentially feeds on dogs during each instar,\(^{35,36}\) is capable of establishing in a variety of climates with regard to temperature, relative humidity, and precipitation,\(^{36}\) including indoor facilities.\(^{35}\) As it is able to adapt to various environmental conditions, *R. sanguineus* is cosmopolitan in its...
geographic distribution. Therefore, the recent discovery of *H canis* in domestic dogs in North America is not entirely surprising.

Additional tick species have been reported as potential hosts of *H canis* in other geographic locations. Oocysts have been identified in *Haemaphysalis longicornis* and *Haemaphysalis flava* collected from dogs naturally infected with *H canis* in Japan. A molecular survey of organisms in wild-caught *Ixodes ricinus* in Luxembourg revealed *H canis* DNA in an unfed adult female. In Brazil, an adult *Amblyomma ovale* collected from a naturally infected dog was reported to contain *Hepatozoon* spp oocysts. Sporozoites liberated from these oocysts were injected into an uninfected dog intraperitoneally and circulating gamonts were observed 84 days after inoculation. Another study demonstrated transstadial transmission of *H canis* by *A ovale* to susceptible dogs, implicating *A ovale* as a definitive host and vector of *H canis* in parts of South America.

Although *Hepatozoon* spp oocysts have been recognized in feeding *Amblyomma maculatum* removed from canids in enzootic areas of ACH, such reports are scarce, and the species of parasites were not determined. However, *A maculatum* (Fig. 2) has experimentally been demonstrated to be an excellent definitive host of *H americanum*, while other common tick species in ACH enzootic areas, including *R sanguineus*, *A americanum*, and *Dermacentor variabilis*, have empirically been refractory to infection. Experiments characterizing the development of *H americanum* in *A maculatum* infected via blood meal acquisition have revealed transstadial maintenance in the tick from larvae to nymph, nymph to adult, and larva to adult. Molted cohorts are demonstrated to harbor sporulated oocysts infective to canine hosts after approximately 33 to 42 days postrepletion in the majority (96% to 99%) of those dissected. Intermittent microscopic examination of experimentally infected ticks has shown evidence of oocyst formation occurring within gut cells of tick hosts. Experiments assessing transovarial transmission of *H americanum* in *A maculatum* have not been reported; this route is not suspected, as it has not been documented in other known definitive hosts of *Hepatozoon* spp.

In the United States, *A maculatum* was traditionally endemic in states bordering the Gulf Coast and several states bordering the Atlantic coast including Georgia, Florida, and the southern portion of South Carolina. However, current data report establishment of the Gulf Coast tick in states farther inland including Oklahoma, Kansas,

**Fig. 2.** *Amblyomma maculatum*, the Gulf coast tick, dorsal view of adult female (left) and male (right) (magnification ×0.8). Notice the diffusely ornate scutum, long palps, and rectangular basis capituli (arrows).
Arizona, Arkansas, Missouri, Indiana, Kentucky, and Tennessee and additional states along the Atlantic coast including Maryland, Virginia, and West Virginia. A maculatum is also documented in Central and South American regions that border the Gulf of Mexico and Caribbean Sea including Mexico, Guatemala, Belize, Nicaragua, Honduras, Costa Rica, Colombia, Venezuela, and parts of Ecuador and Peru, although recent evaluations of historical records in these regions from the past 50 years indicate that Gulf Coast ticks had sometimes been confused with Amblyomma triste. No confirmed reports of H americanum infections have occurred in South America.

Unlike with H canis, other invertebrate definitive hosts of H americanum have not been implicated. Prior to 2008, reports of ACH generally correlated with the geographic distribution of A maculatum in the United States. Newly reported cases of ACH in areas where A maculatum is not established are thought to be instances of patient relocations from confirmed H americanum enzootic areas.

TRANSMISSION OF H CANIS AND H AMERICANUM TO CANID INTERMEDIATE HOSTS

It is conventionally accepted that most Hepatozoon spp infections are acquired by the consumption of invertebrate hosts carrying sporulated oocysts (Fig. 3) of parasite. This may occur when vertebrates ingest invertebrates as sustenance, while grooming self or companions, or accidentally during predation and/or scavenging. The preponderance of knowledge regarding H canis infections has been gleaned from observations in naturally infected domestic dogs. Canid intermediate hosts are thought to primarily become infected by ingesting R. sanguineus ticks that contain H canis oocysts. However, monozoic cysts of H canis have been reported in the spleens of naturally and experimentally infected dogs that are morphologically similar.
to cystozoites, which are arrested zoites encysted in tissues of documented paratenic hosts of other *Hepatozoon* species, that are infective to intermediate hosts ingesting them.\(^1\,^2\,^47\) In dogs infected with *H canis*, these cysts are present in tissues in addition to meronts, which may indicate that dogs serve as both intermediate and paratenic hosts,\(^2\,^48\) although experiments investigating the infectivity of monozoic cysts of *H canis* for dogs consuming them have not been reported.

A tertiary route of congenital transmission, documented initially in natural *Hepatozoon griseisciuri* infections in squirrels, is also reported in *H canis* infections.\(^49\,^50\) Murata and colleagues (1993) monitored *H canis* infections in 6 litters of beagle pups born of 3 naturally infected dams. Fourteen pups comprising 5 litters from 2 infected dams were positive for circulating parasite after 21 to 31 days. Initially, gamonts were present in 0.02% to 0.04% of leukocytes observed, but after several months were present in as many as 3.3%; the rise in parasitemia suggested active merogony and gamont production. Meronts were also observed in the main visceral organs of 1 of 2 pups that died. Additionally, 4 pups whelped from a third infected dam were positive for gamonts 4 weeks after birth, indicating congenital transmission of *H canis* with subsequent parasite establishment and development in these animals.\(^50\)

The primary documented route of *H americanum* transmission to canid intermediate hosts is by the ingestion of infected *A maculatum*.\(^7\,^19\,^46\) However, experiments conducted by Johnson and colleagues in 2008\(^51\) and 2009\(^29\) to establish the susceptibility of several preferred hosts of immature instars of *A maculatum* demonstrated development of cystozoites (Fig. 4) in the tissues of cotton rats (*Sigmodon hispidus*), mice (*Mus musculus*), and New Zealand white rabbits (*Oryctolagus cuniculus*), but not rats (*Rattus norvegicus*), after ingestion of *H americanum* oocysts. The cyst-laden tissues were, in turn, infective to dogs ingesting them. Parasite development and clinical disease in dogs occurred as described in infections resulting from sporozoite ingestion.\(^29\,^41\,^46\) The susceptibility of other hosts to *H americanum* infection with development of cystozoites within these hosts, although experimental, suggests that paratenic hosts for *H americanum* could be a source of infection for

**Fig. 4.** Hematoxylin-eosin–stained section of skeletal muscle from a laboratory-raised New Zealand White rabbit experimentally infected with *Hepatozoon americanum* showing a cystozoite (arrow) (magnification ×100). (Courtesy of Dr Roger Panciera, Oklahoma State University.)
dogs and implies that predation, either of infected prey or prey infested with infected ticks, is a significant epidemiologic factor in natural transmission cycles. Focused wildlife survey studies conducted in ACH enzootic areas in Oklahoma have documented *Hepatozoon* spp infections in trapped rodents and hunted rabbits, but thus far, confirmed natural *H americanum* infections as evidenced by microscopic and molecular data have only been reported in domestic dogs and coyotes. Although speculated to occur, vertical transmission of *H americanum* in naturally infected dogs has not been reported. Most ACH cases are of singly presenting patients residing in rural areas. Patient histories often include behaviors of roaming and predatory tendencies. The youngest reported age of *H americanum* infection is 11 weeks, which is an age allowing enough time from parasite exposure to clinical presentation by either of the 2 established routes of infection. Documented multiple dog outbreaks of ACH have also occurred in rural settings, in animals old enough to roam at will or to be used in recreational hunting pursuits, not in young littermates still relying heavily on their mothers for survival.

A pilot experiment to assess congenital transmission of *H americanum* was conducted at Oklahoma State University in 2008 using a chronically infected, intact female hound. The carrier birthed 8 pups that were monitored for infection weekly for 3 months by complete blood counts, blood smear examination, and PCR of whole blood. None of 7 surviving pups developed clinical signs, laboratory abnormalities, or parasitemia as evidenced by blood film or PCR. One pup died from aspiration pneumonia 4 days after birth; neither histologic lesions nor meronts of *H americanum* were observed in tissues of this pup. At the conclusion of the study, a xenodiagnosis experiment using laboratory-raised *A maculatum* nymphs was performed. None of the molted adult cohorts, dissected approximately 2 months after repletion feeding as nymphs, were found to contain parasite oocysts (Kelly E. Allen, unpublished observations). However, due to only the single dam and litter of pups evaluated, the results of this study cannot be considered definitive evidence for lack of vertical transmission in *H americanum* infections. The timing of infection, whether before or during pregnancy, the stage of parasite by which infected, whether sporozoites or cystozoites, and the clinical phase of disease, whether acute or chronic, may be factors influencing the occurrence of transplacental transmission of *H americanum* in naturally infected dogs.

**H CANIS AND H AMERICANUM TISSUE TROPISM AND DEVELOPMENT WITHIN CANINE HOSTS**

Ingested *Hepatozoon* spp oocysts within tick hosts likely rupture during canid mastication or when introduced into the stomach. It remains unclear whether *Hepatozoon* spp sporozoites released from sporocysts in the canine alimentary tract penetrate the gut lining and migrate to target organs or if they are engulfed by phagocytic cells and carried hematogenously to tissues. Typical sites of merogony in *H canis* infections include bone marrow, lymph nodes, and spleen. In a study conducted by Baneth and colleagues (2007), 2 morphologically distinct populations of meronts were observed in the bone marrow of experimentally infected dogs after 26 days. One form contained only 2 to 4 large zoites, termed macromerozoites, randomly arranged within the meront. The role of macromerozoites in *H canis* infections remains to be elucidated, but they are documented to give rise to micromerozoites and perpetuate merogony in other species of *Hepatozoon*. The second type of meront contained 20 to 30 smaller zoites arranged in a “wheel-spoked” configuration (Fig. 5) similar to that documented in other species of *Hepatozoon*. These zoites, termed micromerozoites, were thought to be the
progenitors of gamonts. Mature gamonts of *H canis* in experimentally infected dogs can be observed in peripheral neutrophils 4 weeks after infection. *H canis* infections are often associated with high levels of parasitemia, with gamonts sometimes reported in as many as 100% of neutrophils on blood smears.

In *H americanum* infections, meronts (Fig. 6) are found within canine host cells, likely monocytes, principally located between individual fibers of skeletal and cardiac muscle tissues as soon as 3.5 weeks after exposure. Maturing meronts of *H americanum* do not have a characteristic “wheel-spoked” arrangement of zoites but, rather, exhibit blastophore formation and appear to transform host cells. In

![Fig. 5](image1.png)

**Fig. 5.** Hematoxylin-eosin-stained section of liver from a naturally infected, field-trapped, cotton rat showing a mature, “wheel spoke” meront of a *Hepatozoon* sp (magnification ×40). (Courtesy of Dr Roger Panciera, Oklahoma State University.)

![Fig. 6](image2.png)

**Fig. 6.** Hematoxylin-eosin-stained section of skeletal muscle from a dog containing an “onion skinned,” early meront of *Hepatozoon americanum* (magnification ×40). (Courtesy of Dr Roger Panciera, Oklahoma State University.)
histologic preparations of muscle tissue, parasitized cells are surrounded by concentric strata of a mucopolysaccharide-rich material reminiscent of onion skin layers.\textsuperscript{12,33} The lesions are aptly termed “onion skin” cysts (Fig. 7).\textsuperscript{12,55,58} Over time, meronts overtake and rupture host cells, thereby liberating merozoites that breach degenerating cyst walls. Merozoites incite local influxes of inflammatory cells that often progress to granulomata (Fig. 8).\textsuperscript{12,55,59} Distinct populations of macromerozoites and micromerozoites as are seen in $H$ canis infections have not been observed in $H$ americanum infections.\textsuperscript{28,55,58} It is hypothesized that some merozoites develop into gamonts after invading new leukocytes while others distribute hematogenously to new sites and continue to reproduce asexually.\textsuperscript{9,12,55,59} Gamonts (Fig. 9), usually

Fig. 7. Hematoxylin-eosin–stained section of skeletal muscle from a $Hepatozoon americanum$–infected dog containing a pyogranuloma (magnification $\times 40$). (Courtesy of Dr Roger Panciera, Oklahoma State University.)

Fig. 8. Giemsa-stained peripheral blood smear from a $Hepatozoon americanum$–infected dog demonstrating a gamont in a peripheral neutrophil (arrow) (magnification $\times 100$).
present in less than 0.1% of circulating white blood cells, are observable on blood smears as soon as 4 to 5 weeks after exposure to *H americanum* zoites. However, they are primarily found during the acute stage of disease.\textsuperscript{12,29}

**CLINICAL PRESENTATIONS AND DIAGNOSIS OF CANINE HEPATOZOOONOSIS**

Disease associated with *H canis* infection may range from subclinical and chronic, especially in the absence of concurrent infections, to severe and life-threatening.\textsuperscript{2,34} Severity of disease tends to correlate with patient immune status, which may be impacted by age, genetic disorder, immune therapy, or coinfection with another etiologic agent such as *Ehrlichia canis*, *Leishmania canis*, *Babesia canis*, and *Toxoplasma gondii*.\textsuperscript{2,13,34,60–62} In rare patients with overt disease, symptoms including fever, anemia, lethargy, anorexia, and depression may be observed.\textsuperscript{2,13,34} *Hepatozoon canis* infections are classically diagnosed by microscopic observation of gamonts in blood films, which sometimes are incidental findings.\textsuperscript{2,7,20} Polymerase chain reaction (PCR) methods have recently been developed to detect parasite DNA in peripheral blood.\textsuperscript{4,6,63,64}

In experimental *H americanum* infections, dogs often present with symptoms of ACH 4 to 5 weeks after ingesting parasite oocysts.\textsuperscript{33} Salient clinical features of ACH include fever, lethargy, copious mucopurulent ocular discharge, pain and reluctance to move, altered gait, and muscle atrophy.\textsuperscript{7,9,13,18,33} Laboratory findings often reveal neutrophilic leukocytosis, which may be profound, and anemia.\textsuperscript{7,13,33} In severe cases, symmetric periosteal bone proliferation, particularly of the long bones, is evident on radiographs (Fig. 9).\textsuperscript{7,13,33,65} Dogs infected with *H americanum* may exhibit waxing and waning courses of clinical disease over time, with clinical relapses attributed to the periodic release of merozoites from tissue meronts and associated inflammation.\textsuperscript{7,18,59} Although chronically infected animals have been reported, ACH patients often die within 12 to 24 months without supportive therapies.\textsuperscript{7,18,19}

![Fig. 9. Radiograph of the hindlimb of a dog with chronic periosteal hypertrophic proliferation commonly detected in *Hepatozoon americanum*-infected dogs. The thickening of the periosteum is most evident in the femur (arrows). (Courtesy of Dr Robert Bahr, Oklahoma State University.)](image-url)
Clinical signs, blood count abnormalities (particularly neutrophilia), observation of rare gamonts in blood smears, and characteristic osteal lesions on radiographs are findings that often lead to a diagnosis of ACH. Muscle biopsy, although invasive, is considered the gold-standard method for achieving a definitive diagnosis, as parasite or parasite-induced lesions can readily be observed in histopathologic stained sections of the biopsied sample. PCR methods have been developed for detecting circulating Hepatozoon spp but may lack sensitivity in H. americanum infections due to low levels of parasitemia.

TREATMENT APPROACHES FOR CANINE HEPATOZOONOSIS

Hepatozoon canis infections are most commonly treated with imidocarb dipropionate twice monthly, administered subcutaneously at 5 to 6 mg/kg, until gamonts are no longer evident in patient blood smears for 2 to 3 consecutive months. The mechanism of action of this compound is not well understood. Although clinical improvement of patients may occur, this drug does not clear H. canis at its currently recommended dose. Hepatozoon canis DNA is detectable in peripheral blood by PCR for weeks following treatment end, even though gamonts have not been microscopically observable on blood or buffy coat smears for several months. Still, clinical signs due to H. canis infection can be well controlled in many patients with this drug compound, although relapses may occur.

Currently, the Companion Animal Parasite Council (CAPC) (www.capcvet.org) recommends presenting ACH patients be treated with either a triple combination of trimethoprim-sulfadiazine (15 mg/kg bid), clindamycin (10 mg/kg tid), and pyrimethamine (0.25 mg/kg once daily) or ponazuril (10 mg/kg bid) for 14 days followed by 2 years of twice-daily decoquinate administration (10 to 20 mg/kg). Decoquinate appears to prevent or delay clinical relapse by arresting merogony. Supplemental nonsteroidal anti-inflammatory drugs (NSAIDs) may be given for fever and pain control.

Triple therapy with trimethoprim-sulfadiazine, clindamycin, and pyrimethamine (TCP) is aimed at inhibiting parasite folic–folinic acid metabolism and is used to treat toxoplasmosis in dogs and cats. Decoquinate is classified as a coccidiostat, but in higher concentrations it is coccidioidal and targets parasite mitochondria. It is commonly used as a preventative of coccidiosis in chickens, sheep, goats, and rabbits. Combined TCP and decoquinate treatment was evaluated in naturally infected dogs in a study conducted by Macintire and colleagues. Although this treatment regimen is not curative, it does extend life expectancy and improve quality of life for many ACH patients. Should clinical relapse occur, it is recommended that TCP or ponazuril treatments be repeated and again followed by long-term decoquinate administration.

Ponazuril (toltrazuril sulfone), a recommended alternative to TCP in the treatment of ACH, is approved by the US Food and Drug Administration solely for the treatment of equine protozoal myeloencephalitis (EPM) caused by Sarcocystis neurona. However, ponazuril is widely used as an effective treatment of Cystoisospora spp infections in young dogs and cats as well as a preventative of coccidiosis in chickens. Ponazuril has been shown to inhibit development of other tissue-cyst forming protozoans including Toxoplasma gondii and Neospora caninum in mice and in vitro systems. Although a currently accepted alternative to TCP treatment of ACH patients, ponazuril had not been evaluated experimentally in H. americanum infections for clinical sign alleviation or clearance of parasite until recently. A pilot trial was conducted by Allen and colleagues (2010) to assess the efficacy of ponazuril as a stand-alone 4-week treatment for ACH. Although extended treatment with 10 mg/kg
ponazuril twice daily for 4 weeks in combination with NSAID administration (2.2 mg/kg carprofen) for pain amelioration of acute clinical signs, parasite clearance was not achieved. Parasites were detected in both the treated dog and a positive control dog by histopathology and PCR 43 weeks postexposure.72

GENETIC CHARACTERIZATION AND PHYLOGENETIC RELATIONSHIPS OF *HEPATOZOOON* spp IN THE UNITED STATES

In addition to canids, a broad range of vertebrate hosts are reported to be infected with *Hepatozoon* species worldwide, with over 300 species observed and named in poikilotherms, other mammals, and some birds.1,2,8 To date, there are 29 species of *Hepatozoon* reported from North America, of which 24 are in snakes.73–78 *Hepatozoon* species reported in mammals include *H. muris* in rats (*Rattus norvegicus*),79 *Hepatozoon procyonis* in raccoons (*Procyon lotor*),80–82 *Hepatozoon griseisciuri* in grey squirrels (*Sciurus carolinensis*),83 *H. americanum* in domestic dogs and coyotes,12,13,31 and, more recently, *H. canis* in domestic dogs.3–5 Undetermined species have been reported in a domestic cat (*Felis catus*),84 bobcats (*Lynx rufus*), and ocelots (*Leopardus pardalis*).84,85

With discoveries of alternate transmission routes of some *Hepatozoon* spp, the utilization of obligate paratenic hosts by several *Hepatozoon* spp, and the abilities of other *Hepatozoon* spp to infect experimental facultative transport hosts, genetic characterization has become an important criterion in the complete description of established *Hepatozoon* species and the proposition of novel species.6,17,52,86 There are approximately 200 *Hepatozoon* sequences listed in the National Center for Biotechnology Information database (NCBI), GenBank, with less than 20 sequences collected from North American animals. Prior to 2007, only *Hepatozoon* sequences obtained from domestic dogs were available in the NCBI database.6,17,87 Since then, 1 sequence collected from cotton rats (*Sigmodon hispidus*), 1 sequence collected from white-footed mice (*Peromyscus leucopus*),52 6 additional sequences from domestic dogs (*Canis familiaris*),3 1 sequence from a cottontail rabbit (*Sylvilagus floridanus*), 1 sequence from a swamp rabbit (*Sylvilagus aquaticus*),46 and 1 sequence (GU344682) from a turkey vulture (*Cathartes aura*) have been contributed to the database. The sequences from 6 additional dogs contributed to GenBank by Allen and colleagues in 2008 demonstrated that the *Hepatozoon* spp organisms infecting domestic dogs in the United States are diverse and provided molecular evidence for the existence of multiple species and strains of canid *Hepatozoon* spp in North America.3

The dearth of *Hepatozoon* spp sequence contributions to GenBank from the United States and the diversity within the data available led to a collaborative effort with other researchers to obtain, compile, and submit *Hepatozoon* spp sequences from known and previously unrecognized hosts.88 An approximate 500–base pair fragment of a hypervariable region of the 18S rRNA gene of *Hepatozoon* spp and some other apicomplexans was amplified from blood or tissues of 16 vertebrate host species from the United States. Phylogenetic analyses and comparison with other *Hepatozoon* spp sequences in GenBank revealed distinct taxonomic groupings. In general, sequences obtained from scavengers and carnivores (1 opossum, 1 gray fox, 4 raccoons, 2 bobcats, 1 domestic cat, coyotes, and domestic dogs) grouped together and sequences from rodents formed a separate cluster. However, interestingly, sequences from wild rabbits were most closely related to sequences obtained from carnivores, and sequence from a boa constrictor was most closely related to sequences within the rodent cluster. These data support recent experimental work identifying predator/prey transmission cycles in *Hepatozoon* spp1,46,47,89 and suggest
this particular transmission pattern may be more common than previously recognized. Additionally, the study possibly elucidated sequence data of Hepatozoon spp previously identified morphologically in grey squirrels and raccoons, provided molecular evidence of H. americanum infections in coyotes, confirming prior suspicions, and documented possible coinfections of coyotes with different strains of H. americanum. A sequence obtained from a domestic cat that was nearly identical (99.4%) to H. americanum indicated that felids are susceptible to H. americanum infection, especially if immunocompromised. Also, a sequence from a gray fox that was most similar to that reported as H. canis indicated the possible susceptibility of North American wild carnivores to H. canis and supported results from 2 previous molecular survey studies in domestic dogs in North America documenting the presence of H. canis in North America.

**SUMMARY**

It is now clear that 2 Hepatozoon species infect domestic dogs in the United States, H. canis and H. americanum. Hepatozoon canis is documented in several states in the Southeast, and H. americanum, previously thought to be a parasite of dogs limited to the south central and southeastern states, is now documented in states in other regions of the country. It is important for veterinarians throughout the United States to understand that although canine hepatozoonosis caused by both organisms is incurable, infections can be well managed with different treatment approaches. It is commonly thought that H. canis is predominantly cycled between R. sanguineus and dogs, whereas recent experimental evidence has demonstrated the ability of H. americanum to use several alternate vertebrate species as paratenic hosts, making it reasonable to consider the possible importance of predator/prey relationships in natural transmission cycles of H. americanum. Hepatozoon spp genetic data from canids and other vertebrates in the United States are diverse, and phylogenetic comparisons of previously and recently documented sequences support the assertion of predator–prey relationships of vertebrate hosts of several Hepatozoon species. Further research is needed to better understand the Hepatozoon organisms cycling in nature in the United States.

**REFERENCES**


