American Canine Hepatozoonosis

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ABSTRACT: American canine hepatozoonosis (ACH) is a debilitating disease caused by Hepatozoon americanum and transmitted by ingestion of oocyst-containing Amblyomma maculatum ticks. A history of incomplete response to common antibiotics, together with physical findings of fever and muscle wasting and laboratory findings of leukocytosis and periosteal proliferation, should prompt testing for ACH. Muscle biopsy and whole-blood polymerase chain reaction are currently the most reliable methods of diagnosis. Treatment consists of a 2-week course of trimethoprim–sulfonamide, clindamycin, and pyrimethamine, followed by a 2-year course of decoquinate. Relapses are common, but prognosis is fair overall.

The cause of American canine hepatozoonosis (ACH) is Hepatozoon americanum, a protozoan transmitted by ingestion of Amblyomma maculatum ticks, or Gulf Coast ticks, that contain the oocysts of the organism (Figure 1). Canine hepatozoonosis was first recognized in North America in 1978 in Texas. All infections in North America were originally thought to be caused by a more virulent strain of Hepatozoon canis, the “Texas strain.” In 1997, however, based on clinical, serologic, and pathologic features; gamont morphology; and inability to experimentally infect Rhipicephalus sanguineus, the brown dog tick and definitive host for H. canis, the cause of ACH was designated as a new species, H. americanum (Table 1). Molecular evidence separating H. canis from H. americanum has since become available. It is now well established that ACH in North America is caused by H. americanum.

GEOGRAPHIC DISTRIBUTION AND EPIDEMIOLOGY

ACH has been reported in Texas, Louisiana, Alabama, Mississippi, Oklahoma, Georgia, Tennessee, and Florida. The geographic distribution of H. americanum is directly related to the distribution of the Gulf Coast tick, which is the obligate definitive host and the source of infective oocysts of the protozoan. This tick, which was once confined to the Texas Gulf Coast, has now expanded its range as far north as southern Kansas and Kentucky. The transport of ACH to nonendemic areas may have occurred via widespread travel of humans and pets. For example, Hurricane Katrina has broadened the distribution of ACH as dogs from the Gulf Coast region were moved throughout the country. These animals may have carried the disease to new areas.

*Personal communication with T. Mark Neer, DVM, MS, DACVIM, Oklahoma State University Center for Veterinary Health Sciences, September 20, 2006.
have been infected with the disease before the hurricane and developed clinical signs after relocation. Therefore, a thorough history is imperative in establishing the potential for exposure to ACH. 

*H. americanum*, or a similar organism, has been identified in domestic dogs and feral coyotes, bobcats, and ocelots in the southern United States. The Gulf Coast tick tends to feed on dogs primarily in its adult form, not as a larva or nymph, making it unlikely that dogs are effective reservoirs or important hosts; dogs are actually considered to be accidental hosts. Because they are not effective reservoirs, infected dogs do not pose an infection risk to other dogs in the area. Under natural conditions, the host range of larval and nymphal Gulf Coast ticks includes birds and small mammals, making these species potential reservoir hosts. The range of species susceptible to infection and serving as natural reservoir hosts is not known.

### TRANSMISSION

The definitive host and vector for *H. americanum* is *A. maculatum*. Repeated attempts to achieve infection of *R. sanguineus, Amblyomma americanum*, or *Dermacentor variabilis* have been unsuccessful. There is one report of transmission via *R. sanguineus*, but others have been unable to duplicate this result. Two other possible vectors (*Haemaphysalis leporispalustris* [Packard], the rabbit tick, and *Ixodes scapularis* Say, the blacklegged tick) have been proposed because these three-host ticks are found in areas endemic for ACH. Further research is required to determine whether these ticks can transmit the hepatozoon organism.

Transmission to intermediate hosts, including the domestic dog and the coyote, is by ingestion of *H. americanum* oocyst-containing Gulf Coast ticks, rather than through tick bites. Transstadial transmission occurs regardless of whether the tick acquires *H. americanum* as a larva or nymph, so dogs can acquire infection by ingesting newly molted nymphs or adult ticks. In utero or congenital (vertical) transmission has been reported with *H. americanum* but has not been documented for *H. canis*. There is no evidence that transmission occurs by consumption of a tissue stage of *H. americanum*, as has been confirmed with *Hepatozoon griseisciuori* in squirrels. There is a single report of a dog fed raw meat containing cysts and pyogranulomas containing merozoites that was...
followed for 9 months and showed neither clinical signs nor hematologic or histologic evidence of infection.6

**LIFE CYCLE**
Following ingestion, sporocysts are quickly released from the fragile-walled oocysts.21 Exposure to bile enhances the liberation of sporozoites from the sporocysts in the dog’s intestines.22 Sporozoites penetrate the intestinal mucosa and are distributed throughout the body to undergo merogony, primarily between fibers of skeletal and cardiac muscle.21 Sporozoites undergo merogony in canine macrophages that secrete mucopolysaccharide in concentric layers surrounding the cell, creating an “onion skin” cyst23 (Figure 2). The latter is a structure unique to, and diagnostic for, ACH. At completion of merogony, the meront ruptures, releasing numerous merozoites and inducing a severe, localized pyogranulomatous reaction as well as systemic illness.17 Free merozoites enter the vasculature and macrophages within the granuloma and either develop into parasitic gamonts or are redistributed in the tissue to undergo repeated asexual cycles.23

When an *A. maculatum* tick feeds on an infected dog, it ingests the gamonts in the peripheral leukocytes. A sexual stage of reproduction followed by asexual multiplication in the tick gut epithelium produces oocysts that contain hundreds of sporocysts, each of which holds 10 to 26 sporozoites.18 As the oocysts develop, most dislodge from the gut epithelium and are freed into the hemocoel (body cavity) of the tick.18,19,22 A gamont ingested by a tick requires 42 days to develop into a mature, sporulated oocyst. Completion of the life cycle in the dog from ingestion of an oocyst to development of circulating gamonts can occur in as few as 32 days under experimental conditions22,24 (Figure 3).

The occurrence of ACH is seasonal, peaking in the warmer months or early fall when dogs are most likely to be exposed to ticks. However, infected dogs may exhibit clinical signs throughout the year as the cycles of asexual reproduction and pyogranulomatous inflammation are repeated.17

**PATHOGENESIS**
The first evidence of ACH appears as early as 3 weeks postexposure, consists of a large zoite-containing macrophage situated between muscle fibers, and is clinically silent.24 As the zoite develops, the host cell produces and extrudes layers of mucopolysaccharides that surround the cell, protecting it from the canine immune system. The inflammatory response to the infection is very limited while the organism is encysted, but when the cyst ruptures, an acute inflammatory response occurs, followed by pyogranulomatous inflammation and clinical illness.17,25 Waxing and waning elevations of body temperature and neutrophilic leukocytosis have been observed 4 to 5 weeks postexposure. Clinical evidence of disease, such as lethargy, bone pain, and ocular discharge, occurs shortly thereafter. Gamonts have been observed in peripheral blood leukocytes 4 weeks after exposure.24 Cysts have been found in muscle biopsy samples for up to 10 years after the initial infection.6

6Personal communication with Roger J. Panciera, DVM, MS, PhD, DACVP, Oklahoma State University Center for Veterinary Health Sciences, October 17, 2008.
Continued reproduction of the organism and release of merozoites lead to waxing and waning clinical signs and relapses after treatment. Chronic infections cause persistent antigenic stimulation and secondary complications, including vasculitis, glomerulonephritis, and amyloid deposition in various organs. Prolonged infections commonly result in progressive weight loss and muscle wasting, with death occurring within 12 months after ingestion. 17,25

**CLINICAL FINDINGS**

Common clinical signs of *H. americanum* infection include an antibiotic-resistant fever of 102.7° to 105.6°F (39.3° to 40.9°C), cachexia, generalized muscle atrophy, depression, hyperesthesia (especially in the paraspinal area), mucopurulent ocular discharge, and gait abnormalities. Gait problems include stiffness, generalized weakness, hindlimb paresis and ataxia, and inability or unwillingness to rise. Hyperesthesia attributed to pyogranulomatous myositis and possibly periosteal reaction may be generalized or localized to the cervical, back, or joint regions. 17,25 Diagnostic differentials for dogs exhibiting these signs include meningitis, diskospondyli-

...the most consistent laboratory abnormality in ACH is an elevated leukocyte count of 20,000 to 200,000/µL, typically consisting of a mature neutrophilia with an occasional mild to moderate left shift. 10 Mild normocytic, normochromic, nonregenerative anemia is a frequent finding, presumably due to chronic inflammation.
Platelet numbers are usually normal to slightly elevated, but marked thrombocytosis has been reported secondary to chronic inflammation. However, if thrombocytopenia is present, concurrent infection with ehrlichiosis, babesiosis, Rocky Mountain spotted fever, or another tickborne disease should be considered. Common serum biochemistry abnormalities are elevations in alkaline phosphatase levels, hypoglycemia, and hypoalbuminemia. The alkaline phosphatase elevation is probably due to periosteal inflammation and reaction. Hypoglycemia, often ranging from 40 to 60 mg/dL, may be the result of in vitro metabolism of glucose by white blood cells. Hypoalbuminemia is attributed to decreased protein intake, chronic inflammation, or protein-losing nephropathy secondary to glomerulonephritis or renal amyloidosis. Serum blood urea nitrogen (BUN) values may be decreased unless significant secondary renal disease is present, causing azotemia. Hypoglycemia, hypoalbuminemia, and a low BUN level mimic liver disease biochemically, but serum bile acid assays are usually normal or only slightly elevated in dogs with ACH. Despite significant myositis, creatinine phosphokinase values are typically normal. Hyperglobulinemia is uncommon, chronic inflammation notwithstanding.

Urinalysis may reveal proteinuria, with an elevated urine protein:creatinine ratio in dogs with secondary glomerular disease. Other diagnostic tests and subsequent findings include electromyography revealing changes consistent with generalized polymyopathy, lymph node aspirates consistent with reactive hyperplasia, and bone marrow cytology findings that include granulocytic hyperplasia and erythroid hypoplasia with a high myeloid:erythroid ratio.

**RADIOPHGRAPIC FINDINGS**

Dogs with ACH may have minimal bone abnormalities or significant changes consisting of disseminated, symmetric, periosteal new bone formation that most frequently involves the diaphyses of the proximal long bones of the limbs (Figure 5). Flat and irregular bones are less commonly affected. The process of periosteal proliferation is histologically detectable as early as 5 weeks after exposure, and the lesions progress rapidly. The pathogenesis of these lesions is unknown, but the disseminated and symmetric distribution of proliferation is more likely attributable to stimulation by humoral factors than to stimulation by local factors. The gross and microscopic bone lesions of ACH are very similar to those seen with hypertrophic osteopathy. In both conditions, the periosteal reaction occurs without cortical destruction, and the lesions primarily affect the diaphyses of long bones. With ACH, the onset and
progression are faster and usually occur in the proximal bones of the limbs. With hypertrophic osteopathy, the proliferation tends to begin in the metacarpals and metatarsals and is typically associated with a primary pulmonary lesion. The pathogenesis of the bone lesions in both conditions is incompletely understood.

Scintigraphy has been used to study the onset and distribution of the early skeletal lesions of ACH. Lesions are bilaterally symmetrical, have high intensity, and occur primarily proximal to the carpus or tarsus on the axial skeleton. These lesions were detected as early as day 67 postinfection, suggesting that bone scintigraphy may be a useful method for identifying ACH bone lesions.

**DIAGNOSTIC CONFIRMATION**

Fewer than 0.1% of peripheral blood leukocytes are infected, making gamont-containing leukocytes few and difficult to find. Gamonts appear within the cytoplasm of leukocytes as pale blue to clear oblong capsules measuring approximately 8.8 × 3.9 µm, with a faintly stained nucleus (Figure 6). Because peripheral blood smears rarely demonstrate the organism,uffy coat smears may be conducted to increase the chance of detecting gamonts.

Muscle biopsy is the most reliable method of confirming the diagnosis of ACH. Tissue samples (2 × 2 cm) are taken from the biceps femoris or semitendinosus muscle. Muscle lesions consist of cysts, pyogranulomas with merozoite-containing macrophages, and myositis. False-negative findings are rare due to the extremely high number of organisms in actively infected dogs. Evaluation of multiple biopsy samples can increase the likelihood of diagnosis.

A real-time polymerase chain reaction (PCR) test for ACH has been developed. This test targets the 18S rRNA gene of *H. americanum* and is capable of detecting ACH when there are as few as 7 organisms/mL of blood. It also distinguishes *H. americanum* from other, similar protozoa such as *H. canis*, *Babesia gibsoni*, and *Babesia canis*. The test is conducted on blood samples treated with EDTA, and the samples can be shipped by regular mail.

An ELISA to identify serum antibodies to ACH has been developed at the Auburn University Molecular Diagnostics Laboratory. For more information, please visit http://www.vetmed.auburn.edu/index.pl/molecular_diagnostics.

**PATHOLOGIC FINDINGS**

At necropsy, cachexia and muscle atrophy are consistent gross findings in dogs chronically infected with ACH, along with thickened, roughened bone surfaces. Pyogranulomas may appear as 1- to 2-mm, white to tan foci scattered throughout muscle and other tissues. Pyogranulomas, cysts, and meronts can be visualized microscopically in skeletal and cardiac muscle and may be found in adipose tissue, lymph nodes, intestinal smooth muscle, and spleen, skin, kidney, salivary gland, liver, pancreas, and lung tissue. An immunohistochemical staining procedure using polyclonal antibodies to sporozoites of *H. americanum* has been developed to identify muscle cysts. Various organs exhibit vascular changes that include fibrinoid degeneration of vessel walls, mineralization and proliferation of vascular intima, and pyogranulomatous vasculitis. Renal lesions are common, including focal pyogranulomatous inflammation with mild glomerulonephritis, lymphoplasmacytic interstitial nephritis, mesangioproliferative glomerulonephritis, and, occasionally, amyloidosis. The spleen, lymph nodes, small intestine, and liver may also contain amyloid deposits. Pulmonary congestion, splenic coagulative necrosis, lymphadenomegaly, and congestion of the gastric mucosa are less common.

**TREATMENT**

Remission of clinical signs can be achieved quickly in most cases by initiating treatment with trimethoprim–sulfonamide, clindamycin, and pyrimethamine (TCP combination) for 14 days (Table 2). Although the
clinical response to treatment can be striking, relapse often occurs in 2 to 6 months when this treatment is used alone.\textsuperscript{29} Decoquinate, a coccidiostat, has been found to reduce relapses. It is ineffective in resolving active disease but can arrest the development of merozoites as they are released from mature meronts. Dogs with ACH that experience relapse should be treated with the TCP combination for 14 days, after which decoquinate should be reinitiated. The optimum duration of decoquinate treatment is debatable, and current recommendations include conducting whole-blood PCR testing every 3 to 6 months and discontinuing the drug once the test result is negative.\textsuperscript{4} Decoquinate is available as a feed additive in 50-lb bags at a concentration of 27.2 g/lb of premixed powder. The powder can be mixed into moist food at a dose of 0.5 to 1 tsp/10 kg of body weight.\textsuperscript{33}

Treatment of ACH can be discouraging because no therapy can eliminate the tissue stages of the organism. Relapses are frequent, and the disease’s natural relapsing–rminating cycle can make treatment success difficult to evaluate. Relapses result from continued release of merozoites into muscle and other tissues as meronts undergo replication and development. Current treatments cannot penetrate host cells to arrest the development of encysted meronts. Relapses increase the likelihood of long-term complications such as glomerulonephropathy, amyloidosis, vasculitis, and chronic cachexia.\textsuperscript{17,25}

Palliative treatment with NSAIDs may provide immediate relief of fever and muscle pain and can be initiated concomitantly with TCP therapy.\textsuperscript{17,25} Imidocarb dipropionate has been suggested as a treatment for ACH, but it does not clear the encysted stage.\textsuperscript{33} Toltrazuril, a coccidiostat, has induced excellent initial clinical responses in dogs with ACH but cannot completely eliminate the parasites, and remission was transient in most dogs.\textsuperscript{9} Toltrazuril is currently not available in the United States.

### PROGNOSIS

In the past, the prognosis for dogs with ACH was considered guarded to poor. Many dogs died or were euthanized due to the severity of their clinical signs. The use of TCP combination therapy followed by daily decoquinate administration has markedly improved this picture. Relapses are now less severe and less frequent, with a lower occurrence of glomerulonephritis and amyloidosis.\textsuperscript{17,25} Researchers have found the 2-year survival rate for dogs that received the TCP combination therapy plus daily decoquinate to be greater than 84%, compared with 12.5% for dogs that received TCP combination therapy alone.\textsuperscript{33}

### PREVENTION

Tick control is the mainstay of prevention for ACH and should consist of effective preventive and acaricidal treatments and regular examination to remove ticks. Environmental control of ticks is also necessary, so yards and outdoor kennels should be sprayed routinely. It is also prudent to refrain from feeding dogs raw meat or organs of wildlife from endemic areas. Although this route of infection has not been documented for ACH, it can occur with other species of *Hepatozoon*.\textsuperscript{17,25}

### CONCLUSION

With dogs being redistributed throughout the United States as a result of recent hurricanes, it is incumbent on clinicians to maintain a high index of suspicion for ACH, regardless of where they practice. Likewise, if *H. americanum* appears to be spreading through the area during the summer months, clinicians should warn clients and educate them about preventive measures.

### REFERENCES


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**Table 2. Current Treatment Protocol for American Canine Hepatozoonosis**

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dosages</th>
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<tbody>
<tr>
<td>Trimethoprim–sulfonamide</td>
<td>15 mg/kg PO q12h for 14 days</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>10 mg/kg PO q8h for 14 days</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>0.25 mg/kg PO q24h for 14 days</td>
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<tr>
<td>Decoquinate</td>
<td>10–20 mg/kg PO q12h for 2 years</td>
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</tbody>
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\textsuperscript{4}Personal communication with Douglass K. Macintire, DVM, MS, DACVIM, DACVECC, College of Veterinary Medicine at Auburn University, March 23, 2007.


12. The most consistent laboratory finding in dogs infected with *Amblyomma maculatum*, the Gulf Coast tick.


**CE TEST**

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1. **Dogs become infected with *Hepatozoon americanum* through**
   a. consumption of raw meat.
   b. the bite of *Amblyomma maculatum*, the Gulf Coast tick.
   c. the bite of *Rhipicephalus sanguineus*, the brown dog tick.
   d. ingestion of *Amblyomma maculatum*, the Gulf Coast tick.

2. **Exposure to bile enhances the liberation of**
   a. oocysts
   b. sporocysts
   c. sporozoites
   d. gamonts

3. **The most common clinical sign(s) of ACH include**
   a. fever.
   b. mucopurulent ocular discharge.
   c. muscle wasting.
   d. all of the above

4. **The most consistent laboratory finding in dogs with ACH is**
   a. ALP elevation.
   b. leukocytosis.
   c. thrombocytosis.
   d. hypoglycemia.
5. Periosteal proliferation associated with ACH most closely resembles which disease process?
   a. hypertrophic osteopathy
   b. hypertrophic osteodystrophy
   c. osteomyelitis
   d. neoplasia

6. The major target tissue of *H. americanum* is
   a. the spleen.
   b. skeletal muscle.
   c. smooth muscle.
   d. the lymph nodes.

7. What is the most accurate method for confirming the diagnosis of ACH?
   a. identification of gamonts in leukocytes in peripheral blood smears
   b. muscle biopsy
   c. PCR
   d. ELISA

8. *H. americanum* parasitizes which type of canine host cell?
   a. macrophage
   b. neutrophil
   c. eosinophil
   d. basophil

9. The current recommended treatment protocol for ACH is
   a. imidocarb dipropionate.
   b. toltrazuril.
   c. decoquinate.
   d. trimethoprim–sulfonamide, clindamycin, pyrimethamine, and decoquinate.

10. When used to treat ACH, decoquinate is
    a. capable of killing mature meronts.
    b. usually administered on an empty stomach.
    c. ineffective against active infections.
    d. given twice daily for 6 months.