Treatment of *Hepatozoon americanum* Infection: Review of the Literature and Experimental Evaluation of Efficacy*

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**CLINICAL RELEVANCE**

There is no labeled treatment for dogs with American canine hepatozoonosis (ACH), but the drug therapies discussed in this article, although not rapidly curative, may be successful in alleviating acute clinical signs, prolonging life, reducing the number of clinical relapses, and enhancing quality of life. This article also describes a pilot trial conducted to assess the efficacy of a novel treatment approach with ponazuril as a stand-alone parasiticide administered for 4 weeks without follow-up decoquinate treatment. Although extended ponazuril treatment in combination with NSAID administration did ameliorate acute clinical signs associated with ACH, the parasite was not completely cleared with this treatment protocol alone. Long-term decoquinate therapy remains a critical component of successful treatment of ACH.

**INTRODUCTION**

*Hepatozoon americanum* is an apicomplexan protozoan that causes American canine hepatozoonosis (ACH), a painful disease in dogs in North America.¹ ² Dogs become infected with *H. americanum* by ingesting ticks infected with sporulated oocysts or prey infected with quiescent cystozoite stages of the parasite.³ ⁵ The natural reservoir host(s) have not been clearly identified, but coyotes, rabbits, rodents, and other vertebrate prey species may play a role in maintaining a cycle of infection in nature.³ ⁶ Clinical signs of ACH include fever, mucopurulent ocular discharge, reluctance to move, wasting,

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gait disturbance, and abnormal blood profiles revealing extreme neutrophilia.\textsuperscript{2,5,7–9} Without supportive drug therapy, dogs often worsen in clinical condition and die within 1 to 2 years.\textsuperscript{8}

The only known tick vector of \textit{H. americanum}, \textit{Amblyomma maculatum}, was historically restricted to US states bordering the Gulf Coast, but recent data indicate its establishment in central states as far inland as Iowa and Illinois and in states bordering the Atlantic Coast as far north as Maryland and Delaware.\textsuperscript{10,11} At the same time, the distribution of \textit{H. americanum} infections in domestic dogs, previously considered endemic in the southeastern United States, has expanded to include several south-central, southeastern, midwestern, and mid-Atlantic states. A recent nationwide survey of real-time polymerase chain reaction (PCR) assay results revealed \textit{H. americanum} infections in areas of the country where cases of ACH have not previously been documented.\textsuperscript{12} Domestic travel of pet owners and relocation of rescue dogs have likely contributed to the introduction of \textit{H. americanum} into previously unreported areas. Thus, ACH is becoming an increasingly important disease to the health of domestic dogs throughout the United States. However, despite increasing national recognition of ACH as an emerging disease in the field of veterinary medicine, a rapidly curative treatment has not yet been identified.\textsuperscript{8,9}

No drug therapies are labeled for the treatment of \textit{H. americanum} infection. Patients with ACH are prescribed palliative drug therapies, such as carprofen, to aid in pain relief and parasiticides to control the infection.\textsuperscript{8,9} A study conducted by Macintire et al\textsuperscript{8} assessed the efficacies of toltrazuril and a combination of trimethoprim–sulfadiazine, clindamycin, and pyrimethamine (TCP) in naturally infected, client-owned dogs with histologically confirmed merozoite stages of \textit{H. americanum} in muscle tissue. The dogs were divided into groups that received toltrazuril for 5 days or 10 days, TCP for 14 days without follow-up decoquinate treatment, or TCP for 14 days with follow-up decoquinate treatment. Dogs were followed for up to 2 years. Results of the study indicated that the 14-day TCP treatment period followed by long-term daily decoquinate administration, although not curative, was the drug regimen that often gave the most favorable prognosis.

The Companion Animal Parasite Council (CAPC) currently recommends 14-day initial parasiticide treatments with either a triple combination regimen of TCP or ponazuril (toltrazuril sulfone) alone, followed by a minimum of 2 years of twice-daily decoquinate to prevent relapse. Clinical signs often recur if treatment is not consistently administered as directed.\textsuperscript{8} Data supporting current recommendations for ACH drug therapy protocols are gathered from naturally occurring cases. Although such cases are of clinical value, patient histories almost invariably lack detailed information regarding time, route, and dose of parasite inoculation. Also, clinical follow-up data often depend on pet owner compliance. Convalescing dogs may not be monitored in a controlled setting with consistent clinical evaluation and testing. Thus, it is difficult to broadly assess parasite response to different treatments in naturally infected dogs over time.

In this paper, we review evidence supporting currently recommended treatments for ACH and describe a pilot trial that evaluated a 4-week regimen of oral ponazuril for its ability to limit infection in an experimentally infected dog. For this purpose, one experimentally infected dog was treated with ponazuril and carprofen (for pain), one experimentally infected dog was treated with carprofen only, and one uninfected dog was administered ponazuril and carprofen and served as a negative control. The dogs were evaluated for parasite
presence by observation of clinical signs, muscle biopsy histology, and PCR assay of whole blood and muscle tissue.

### MATERIALS AND METHODS

#### Dogs

Three dogs obtained from a commercial vendor were housed in the animal resources facility at Oklahoma State University for several months before the start of this study. Before experimental infection, whole blood from all three dogs was collected in EDTA and evaluated by complete blood count (CBC) to verify normal blood profiles. PCR assay of whole blood, performed as described elsewhere, was carried out to confirm that all dogs tested negative for *H. americanum* and *Hepatozoon canis*. Muscle biopsy samples, collected under anesthesia, were examined histologically and tested by PCR to ensure that none of the dogs harbored muscle stages of *H. americanum*.

#### Experimental Infection of Dogs

Two of the three dogs used in this study were infected by the administration of approximately 40 mature oocysts harvested from molted adult *A. maculatum* ticks that had fed as nymphs on an experimentally and chronically infected carrier dog. The oocysts were enumerated, suspended in physiologic saline, and mixed with wet, canned dog food. The two dogs were observed to eat the entire serving of dog food containing the oocyst suspensions. The third dog served as a negative control to evaluate drug safety only and was not exposed to oocysts.

#### Sample Collection and Clinical Monitoring

After inoculation of the two dogs, whole blood from all three dogs was collected in EDTA for PCR assay and CBC every 2 weeks for 6 weeks, then weekly for 4 weeks until the infected dogs were observed to display clinical signs of acute disease and tested positive by PCR for DNA of parasites circulating in whole blood. Muscle biopsy samples were collected from all three dogs at 10 weeks postexposure to confirm the presence of established parasite stages in muscle tissue of experimentally infected dogs by histopathology and PCR before beginning treatment. All three dogs were evaluated weekly by PCR, CBC, and observation of clinical signs during the treatment period and at 2, 3, and 8 weeks after treatment end. The uninfected negative-control dog was then released from the study. The infected dogs were evaluated by PCR assay of whole blood on seven more occasions and a final CBC analysis conducted 15 weeks after ponazuril treatment end. The dogs were euthanized and necropsied 43 weeks after experimental infection, and blood and muscle tissue samples were harvested for testing by PCR.

#### Treatments

One of the two experimentally infected dogs was treated with 10 mg/kg ponazuril (Marquis, Bayer HealthCare LLC), donated by Bayer HealthCare LLC, administered orally every 12 hours for 4 weeks. Ponazuril treatment was withheld in the second experimentally infected dog, which served as a control to monitor disease progression without parasiticide intervention. Both infected dogs were given 2.2 mg/kg carprofen (Rimadyl, Pfizer Animal Health) to control pain during the ponazuril treatment period and until the study ended. The third, uninfected dog served as a drug safety control and was administered treatments identical to those of the infected, ponazuril-treated dog.

#### COMPLICATIONS

One of the dogs had several epileptic seizures at the beginning of the study after initial sedation for radiography. The condition was noted before experimental infection by oocyst admin-
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**Treatment**

- **Ponazuril and carprofen**
  - 11: 102.5, 9577, +
  - 12: 100.8, 9720, +
  - 13: 102.3, 9856, +
  - 14: 100.6, 6844, +

- **Carprofen**
  - 15: 101.1, 7888, +
  - 16: 100.3, 6954, +
  - 17: 101.1, 7888, +
  - 18: 101.2, 5472, -
  - 19: 8384, -
  - 20: 18,056, -

- **No treatment**
  - 21: 100.7, 4312, -
  - 22: 100.7, 5452, -
  - 23: 102.3, 6222

**PCR** = polymerase chain reaction, **WPE** = week postexposure.
istration and before administration of any treatment and thus was deemed incidental. The dog was managed daily with anticonvulsive medications. No more seizures were observed, and the dog later served as the infected, untreated (no ponazuril) control in the study.

RESULTS
Clinical Signs Displayed by Experimentally Infected Dogs

The infected dog designated to receive ponazuril treatment began to display early signs of ACH 7 weeks after inoculation with *H. americanum* oocysts. During the time between first clinical signs displayed and ponazuril treatment, this dog’s clinical signs consistently included malaise, inappetence, and mucopurulent ocular discharge. Elevated body temperatures were noted for this dog in weeks 7 through 9 postexposure (Table 1). At 10 weeks postinoculation, the dog was observed to have a stiff and painful gait characteristic of dogs infected with *H. americanum*.

After 1 week of treatment with ponazuril and carprofen, this dog’s fever subsided and other signs of disease (ocular discharge, inappetence, stiffness) were observably less pronounced. Signs associated with acute ACH did not return during the 29-week monitoring period after ponazuril treatment ended.

The infected dog that did not receive ponazuril and carprofen, this dog’s fever subsided and other signs of disease (ocular discharge, inappetence, stiffness) were observably less pronounced. Signs associated with acute ACH did not return during the 29-week monitoring period after ponazuril treatment ended.

The infected dog that did not receive ponazuril treatment began to display early clinical signs of ACH 10 weeks postinoculation. Signs included mucopurulent ocular discharge and neutrophilia, but not malaise, inappetence, or stiffness in gait. This dog’s body temperature was only marginally elevated in the seventh week postinoculation (Table 1). The dog was administered carprofen only and never developed more severe signs of ACH.

The unexposed dog designated to serve as the drug safety control showed no clinical signs of disease during the interim between the inoculation of infected dogs and start of treatments. The ponazuril and carprofen administered had no apparent ill effect on the health of this dog during the treatment period. After treatment was ceased, the dog remained healthy through its remaining time in the study.

Neutrophil Counts

The infected dog treated with ponazuril showed slight neutrophilia 7 weeks after exposure to oocysts (Table 1). This dog’s neutrophil count continued to rise for the next 3 weeks, reaching 27,378 cells/µL at 10 weeks postinoculation, the week ponazuril and carprofen treatment commenced. After 1 week of treatment and for the next seven sampling times over a period of 14 weeks, this dog’s neutrophil count was within the normal reference range (2060 to 10,600 cells/µL). However, 15 weeks after ponazuril treatment cessation (29 weeks after experimental infection), marked neutrophilia (18,056 cells/µL) was again observed.

The infected dog treated only with carprofen had a neutrophil count of 13,970 cells/µL 9 weeks after exposure. At the time of ponazuril treatment of the other infected dog (week 10), this dog’s neutrophil count had increased to 21,725 cells/µL and remained elevated for the next 4 weeks. Neutrophil counts decreased to within normal range after 4 weeks of carprofen treatment but were found to be high for the next three sampling intervals over a 7-week period. After 19 weeks of carprofen treatment (29 weeks after experimental infection), this dog’s neutrophil count was 23,408 cells/µL.

The unexposed control dog maintained normal neutrophil counts throughout the study.

Biopsies

Muscle biopsy samples taken from all three dogs 1 week before inoculation were negative for *H. americanum* by PCR and negative for parasite stages and characteristic inflammatory
lesions by histology. Samples were taken again at 9 weeks postinoculation (1 week before treatment commencement) and confirmed parasite presence in the experimentally exposed dogs and parasite absence in the uninfected control dog. Muscle tissue samples taken at necropsy (43 weeks postinoculation) from the two experimentally infected dogs were tested by PCR and examined histologically. Parasite was detected by PCR and was observed microscopically in samples collected from both dogs.

**Polymerase Chain Reaction Assay of Whole Blood**

The first positive PCR assay result in the infected dog designated for ponazuril treatment was obtained 2 weeks after inoculation (Table 1), but parasitemia subsequently decreased to undetectable levels for 4 weeks. A positive PCR result was again observed for this dog at 7 weeks postexposure. For the next 7 consecutive weeks, including the 4 weeks of ponazuril treatment, this dog tested positive for circulating parasite. Positive PCR results were observed for this dog twice more between treatment cessation and study end and on the final day of the study, 43 weeks after experimental exposure to *H. americanum* oocysts.

The infected dog treated with carprofen only also had a positive PCR assay result 2 weeks after inoculation. Subsequent PCR results were negative until 7 weeks postinfection; they then remained (intermittently) positive for 15 weeks of the study. However, whole blood from this dog tested negative for circulating parasite on the final day of the study.

The unexposed control dog had negative PCR assay results for *H. americanum* infection throughout the entire time used in the study.

**DISCUSSION**

Current therapies for ACH often result in prolonged survival and relief of clinical signs in carefully managed patients. However, recommended parasiticides administered for a period of 14 days (as indicated) do not clear muscle cyst stages of *H. americanum*. Ponazuril is a major metabolite of toltrazuril, which is a coccidiosis preventive widely used in poultry. Ponazuril (toltrazuril sulfone) is approved by the US Food and Drug Administration for the treatment of horses with equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona*. *S. neurona*, another oocyst-forming protozoan, disseminates systemically after ingestion and sequesters in neural tissue similar to the manner in which ingested *H. americanum* disseminates in its intermediate host and localizes in muscle tissue. In horses with EPM, the recommended ponazuril dose of 5 mg/kg administered orally for 28 days results in neurologic improvement in a large number of patients. Although *S. neurona* may not be cleared from equine neurologic tissue, infections can often be controlled by ponazuril administration after clinical signs are evident or if ponazuril is administered prophylactically.

Ponazuril is labeled only for use in horses with EPM, but it is highly effective against apicomplexans other than *S. neurona*. In vitro and in vivo studies have demonstrated its inhibitory effect on *Toxoplasma gondii* development. Studies in cell culture systems also indicate that *Neospora caninum* is vulnerable to ponazuril treatment. Ponazuril is often used as an off-label treatment for coccidiosis in dogs and cats. It may also be used as an alternative to TCP in initial treatment of ACH. Although no clear advantage to the patient is reported for ponazuril compared with TCP treatment, the treatment regimen is considerably easier for pet owners. Rather than the recommended one, two, or three times daily administration of the three compounds in the TCP regimen, ponazuril is administered only twice a day and, as
a paste, can be diluted to the correct concentration and mixed with food.

Ponazuril has proved safe in juvenile dogs treated for coccidiosis at doses as high as 250 mg/kg. Also, to our knowledge, no reported studies have demonstrated that ponazuril is unsafe if administered for prolonged periods. Many researchers believe this compound shows promise as an effective preventive for EPM, toxoplasmosis, and coccidiosis when administered prophylactically. Although we did not evaluate it, the prophylactic activity of ponazuril (if any) could be of value for dogs in areas where they are likely to be exposed to *H. americanum*. However, we speculate that the use of ponazuril after ACH diagnosis is probably not rapidly curative because parasite meront stages are already encysted in muscle tissue. *H. americanum* induces a waxing and waning course of disease, possibly due to repeat parasite merogonic cycles, merozoite release, and merozoite invasion of new muscle and cardiac tissue.

Currently recommended parasiticide compounds may initially control *H. americanum* infections by destroying circulating merozoites, but they are ineffective against parasite stages protected in muscle tissue cysts. It is possible that continued ponazuril treatment, thus far shown to have no deleterious effects on animal health, would continue to control disease in patients with ACH, but prolonged decoquinate administration is already recommended by CAPC for this purpose.

In this study, we demonstrate that *H. americanum* is not cleared in an experimentally infected dog treated with ponazuril for a 4-week period based on PCR assay of whole blood and PCR assay and histologic examination of biopsied muscle tissue. Although clinical signs of ACH resolved in the experimentally infected dog treated with ponazuril and carprofen, carprofen alone may have resulted in clinical improvement in the second experimentally infected dog. Neutrophil counts of both experimentally infected dogs were within the normal reference range after beginning treatment with ponazuril and carprofen or carprofen alone; however, values became elevated in the weeks after ponazuril treatment ended. Given that carprofen was administered from 10 weeks postexposure until the end of study, neutrophil levels of infected dogs were likely not affected by this drug’s administration, although it appears to have alleviated pain associated with inflammatory lesions in muscle tissue. Although clinical *H. americanum* infection did temporarily resolve in the ponazuril-treated dog, the dog’s blood tested positive consistently by PCR for presence of parasite during and after the treatment periods. Blood and muscle tissue from this dog were PCR positive at necropsy, 31 weeks after treatment end.

**CONCLUSION**

The results from this pilot study indicate that ponazuril administration, together with NSAID administration, alleviates clinical signs associated with acute ACH. They also confirmed the necessity of long-term follow-up decoquinate administration as currently recommended. Without continued decoquinate management, ACH patients with chronic disease may relapse; relapse probably results from recurrent parasite merogonic cycles.

**ACKNOWLEDGMENTS**

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**REFERENCES**


