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Efficacy of Azithromycin and Compounded Atovaquone for Treatment of *Babesia gibsoni* in Dogs

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Background: Approximately one-third of dogs confiscated during dogfighting investigations are infected with *Babesia gibsoni*. Traditional management of *B. gibsoni* with polymerase chain reaction (PCR)-screening, treatment with commercially available azithromycin and atovaquone, and PCR testing after 60 and 90 days is costly and impractical for large numbers of dogs at a time.

Hypothesis/Objectives: To assess the efficacy of an alternative protocol in which commercial atovaquone was replaced by compounded medication and PCR monitoring was initiated at 30 days after the end of treatment to decrease the total management time.

Methods: Prospective observational study. Forty-two pit bull-type dogs confiscated as part of an investigation of dogfighting, diagnosed with *B. gibsoni* infection, and judged to be suitable for adoption were treated with azithromycin (10 mg/kg PO q24h) and compounded atovaquone (13.4 mg/kg PO q8h with a fatty meal) for 10 days. PCR testing was repeated at 30 and 60 days after end of treatment if dogs with positive PCR tests at either time were tested at 90 days. Treatment was considered successful; 2 PCR tests 30 days apart were negative.

Results: Treatment was successful in 39 dogs (93%) as defined by 2 consecutive PCR-negative test results 30 days apart. In 38 dogs (90%), PCR results were the same at 30 and 60 days.

Conclusions and Clinical Importance: Use of compounded atovaquone and a reduced monitoring period can reduce costs and holding times without compromising treatment efficacy. This more economical protocol can remove barriers to mass screening and management of *B. gibsoni* infections in dogfighting cases.

Key words: Anemia; Animal shelter; Babesiosis; Dog fighting; Pit bull.

Introduction

Organized dogfighting is a common activity throughout the world. In the United States, this activity is a felony offense at the state and federal level.[1] Hundreds of arrests are made for dogfighting every year, resulting in the confiscation of thousands of dogs. Confiscated dogs are considered evidence and are typically held in animal shelters during the legal proceedings, a process that can take months to complete.[1]

Evolving attitudes about animal welfare and accumulating experience with dogs rescued in dogfighting cases have led to changes in the outcomes for confiscated dogs. Historically, it was common for dogs to be routinely euthanized based on the assumption they would be too aggressive to be rehomed. Therefore, diagnosis and management of infectious diseases were of little value. There is currently a growing trend to assess dogs both medically and behaviorally to determine their suitability for adoption into homes.[2,3] Besides the obvious animal cruelty involved, fighting dogs are at increased risk for several infectious diseases.[3] This creates a potential risk for the spread of disease when other dogs are exposed in dogfights, in animal shelters, and after adoption.

Infection with *Babesia gibsoni* is widespread among fighting dogs and is found almost exclusively among breeds commonly used for fighting or dogs that have been bitten by one of those breeds.[3–13] Prevalence of *B. gibsoni* in dogs confiscated in dogfighting investigations is 34%[9] to 39%[3,9] in 2 reports. The risk of *B. gibsoni* was increased 5.5-fold in pit bull-type dogs with scars on the head and forelimbs.[9] Bite wounds and vertical transmission are believed to be the most common route of infection in the US, whereas transmission by ticks or transfusion of contaminated blood appears to be less common in this country.[3,13,14] Clinical signs of infection can include hemolytic anemia, fever, splenomegaly, and malaise, but many dogs have subclinical infections.[4–6] Relapse of parasitemia can occur after splenectomy or immunosuppressive therapy.

The currently recommended treatment for *B. gibsoni* is administration of azithromycin every 24 hours in combination with atovaquone every 8 hours for 10 days.[13,15] A course of antibiotic treatment for an average-sized 20-kg dog using the human-labeled

Abbreviations:

PCR	polymerase chain reaction
ELISA	enzyme-linked immunosorbent assay

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Location of work: All work was performed at the temporary animal shelter facility housing the confiscated dogs and at the University of Florida.

Previous abstract presentation: Results were presented in part in an abstract at the 2014 ACVIM Forum, Nashville, TN.

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products in 2017 would cost approximately $283–$349. In contrast, replacing human-labeled brand-name[a] or generic[b] atovaquone oral suspension with compounded capsules[c] would reduce the average cost to $140 per dog. Current recommendations for confirmation of treatment efficacy include performance of polymerase chain reaction (PCR) at 60 and 90 days after end of treatment.[13,15] This extended monitoring period is intended to ensure that antimicrobial residues and remnants of nonviable *B. gibsoni* are no longer in circulation at the time of sample collection. In addition, samples collected on 2 or more occasions 30 days apart increase the chance of confirming that treatment resulted in a durable suppression or elimination of organisms rather than a transient suppression of parasitemia.

While the traditional treatment and monitoring protocol might be practical for privately owned pets, the cost and duration can be prohibitive in mass-treatment cases housed in a sheltering facility. The purpose of this project was to evaluate the efficacy of a treatment and monitoring protocol made less expensive by use of compounded atovaquone and of shorter duration by initiating post-treatment monitoring at 30 days rather than 60 days.

Materials and Methods

Animals

A total of 42 pit bull-type dogs confiscated in a federal dogfighting investigation, diagnosed *B. gibsoni* infection, and judged to be suitable for adoption were selected for treatment. Ages estimated based on dentition included 6 juveniles <6 months of age and 36 adults ≥6 months of age. Forty of the dogs originated from 6 different scenes in Alabama, Mississippi, and Texas, and were transported to a temporary shelter in Florida to be held as evidence during the legal proceeding; 2 of the treated dogs were puppies born to a dog that was pregnant at the time of the confiscation. After confiscation, dogs were vaccinated against rabies, *Bordetella bronchiseptica,* distemper virus, adenovirus-2, parainfluenza, and parvovirus according to guidelines for animal shelters.[16] Dogs received treatment for internal and external parasites with fenbendazole and monthly moxidectin/imidacloprid or imidacloprid/permethrin and oral ivermectin according to guidelines of the Companion Animal Parasite Council.[17] Puppies born in the shelter also received pyrantel pamoate and ponazuril. Adult dogs were housed individually in chain-link portable kennels. Puppies were housed with littermates, and nursing dams were housed with litters. *Babesia gibsoni* infections were subclinical in all dogs except one that had an acute hemolytic crisis 2 weeks after intake to the shelter.

Sample Collection and Analysis

Dogs were tested at the time of admission for a panel of infectious diseases using serology, PCR, and fecal analysis as previously described.[3] Initial diagnosis and treatment monitoring for *B. gibsoni* were based on identification of *Babesia spp.* (ssrRNA, AF271082) and *B. gibsoni* (heat shock protein 70, AB248731) in EDTA whole blood samples tested by real-time PCR at a commercial reference laboratory[d] as previously described.[3] In addition, serum was tested for *B. gibsoni* antibodies by enzyme-linked immunosorbent assay (ELISA).[3] Puppies born in the temporary shelter were tested by PCR for *B. gibsoni* at 4 and 8 weeks of age.

Treatment and Monitoring

Dogs were treated with azithromycin (10 mg/kg by mouth every 24 hours) in tablet form (various generic brands) and atovaquone (13.4 mg/kg by mouth every 8 hours with a fatty meal) compounded into capsules[c] for 10 days. Response to treatment was monitored by repeated PCR testing for *B. gibsoni* at 30 and 60 days. Dogs with positive PCR tests at either of those dates were tested again at 90 days. Treatment was considered to be successful if 2 PCR tests 30 days apart were negative.

Results

Of 42 dogs treated for *B. gibsoni*, 37 (88%) were PCR-negative at both 30 and 60 days (Table 1). Two dogs (dogs 15, 21) that were PCR-positive at 30 days became PCR-negative at both 60 and 90 days. Altogether, 39 dogs (93%) met the criteria for treatment success with 2 consecutive PCR-negative test results 30 days apart.

Two of the dogs (dogs 4, 16) that were PCR-negative at 30 days reverted to PCR-positive at 60 and 90 days, and 1 dog (dog 34) was PCR-positive at all 3 time points after treatment. These 3 dogs were considered to be treatment failures. Two were retreated 20 weeks (dog 4) and 14 weeks (dog 34) after the first treatment, respectively. Both dogs were PCR-negative 30 days after the second treatment, with reversion to PCR-positive at the next time point.

Table 1. Results of polymerase chain reaction (PCR) testing for *Babesia gibsoni* after 10 days of treatment with azithromycin and atovaquone.

Site of Origin	Dog Numbers	Juveniles (no.)	Adults (no.)	Pretreatment *B. gibsoni* Antibodies (%)	Pretreatment PCR+ (%)	Post-Treatment PCR + Day 30 (%)	Post-Treatment PCR + Day 60 (%)
1	1–19	2	17	17/17 (100)	19/19 (100)	1/19 (5)	2/19 (11)
2	20–23	0	4	4/4 (100)	4/4 (100)	1/4 (24)	0/4 (0)
3	24	0	1	1/1 (100)	1/1 (100)	0/1 (0)	0/1 (0)
4	25–31	0	7	7/7 (100)	7/7 (100)	0/7 (0)	0/7 (0)
5	32–41	4	6	9/10 (90)	10/10 (100)	1/10 (10)	1/10 (10)
6	42	0	1	1/1 (100)	1/1 (100)	0/1 (0)	0/1 (0)
Total		6	36	39/40 (98)	42/42 (100)	3/42 (7)	3/42 (7)

Detailed results for each dog are available in Table S1.

Only 1 dog had clinical signs that could be directly attributed to *B. gibsoni* infection. This dog (dog 28) suffered a hemolytic crisis in which the PCV decreased from 29% at intake to 14% 2 weeks later. Treatment for *B. gibsoni* was started at the time of emergency blood transfusion, followed by negative PCR tests at 30, 48, and 79 days.

After treatment, dogs were transferred to other humane organizations or adopted, so long-term monitoring was not included as a project objective. However, 5 successfully treated dogs (dogs 6, 10, 14, 15, 18) were available for repeated testing a year after treatment, at which time PCR tests remained negative.

Although the effect of treatment on the offspring of pregnant dogs was not a primary focus of this project, 7 pregnant dogs cared for during the dogfighting case provided an opportunity to observe the outcomes of their litters. Four of the pregnant dogs had positive PCR tests for *B. gibsoni* at the time of intake, and 3 had negative tests. Three of the positive dams (dogs 6, 29, 30) were treated, 1 during the last trimester of pregnancy, and 2 during early lactation. The treated dams gave birth to 22 surviving puppies, all of which were PCR-negative at 4 and 8 weeks of age. Three pregnant dogs with negative PCR tests at the time of intake gave birth to 18 surviving puppies, 16 of which were PCR-negative. Unexpectedly, 2 puppies (dogs 15, 16) born to 1 PCR-negative/antibody negative dam were PCR-positive when tested at 4 weeks of age. After this result, the dam was retested and was found to be PCR-positive. Treatment was successful in 1 puppy (dog 15) and unsuccessful in the other (dog 16).

Discussion

In this abbreviated reduced-cost protocol, 39 of 42 (93%) *B. gibsoni*-infected dogs confiscated in a dogfighting investigation responded to treatment with azithromycin and compounded atovaquone. This is similar to the 82% response rate in dogs treated with azithromycin and a commercially available liquid suspension of atovaquone licensed for use in human patients and monitored by PCR testing for 90 days.[15]

Treatment failures were documented in 3 dogs, including 2 dogs that were treated twice. Reinfection and lack of treatment compliance were unlikely causes in this case as dogs were housed individually indoors, treated for ectoparasites, and cared for by shelter staff. Persistent detection of *B. gibsoni* has been reported in both experimental[18] and natural[15,19] infections treated with azithromycin and atovaquone.

One possible reason for treatment failure is inadequate plasma and tissue levels of medications due to poor absorption or rapid elimination. Pharmacokinetic studies required to document such an occurrence were not performed in this study. Two of the 3 dogs that failed to respond to treatment were puppies. Children younger than 2 years of age can require atovaquone doses that are 2–3 times higher than older children and adults to achieve therapeutic plasma levels.[20] In human patients, several-fold increased bioavailability of atovaquone is associated with taking medication with a fatty meal,[21,22] which is the reason dogs in this study were given a meal with their medication. Reduced bioavailability in humans is associated with simultaneous treatment with certain medications, such as tetracycline, rifampin, and metoclopramide.[22] Neither pharmacokinetic studies of atovaquone nor the optimal drug concentrations to eliminate *B. gibsoni* have been reported in dogs.

Another possibility for treatment failure is that affected dogs carried a medication-resistant strain of *B. gibsoni*. Atovaquone resistance in dogs has been associated with a mutation of the *B. gibsoni* mitochondria cytochrome *b* gene that occurs naturally or can arise during treatment.[19,23,24] Two of the treatment failures occurred in a group of 19 infected dogs originating from the same site; the other 17 dogs from the site were successfully treated. The third treatment failure occurred in a group of 10 infected dogs, 9 of which were successfully treated. Although dogs from the same site likely shared some common exposure risks such as fighting, breeding, lactation, and arthropod vectors, dogfighting also involves the movement of dogs over great distances to interact with unrelated dogs. Thus, it is possible for 1 group of dogs to have multiple introductions of different strains of *B. gibsoni* over time.

The results of PCR testing for *B. gibsoni* performed at 30 days after treatment were similar to those conducted at later times, with only 2 of 40 dogs reverting from a negative to positive status in subsequent testing and 2 progressing to negative status after an initial positive test at 30 days. Although uncommon, false-negative PCR test results at early time points could be due to suppression of organisms by medication residues or to the elimination of susceptible organisms that transiently decreases parasitemia below the limits of detection. False-positive PCR test results could be due to the detection of dead organisms still in circulation. These results suggest that monitoring can be initiated earlier than the previous recommendation of 60 days without a substantial increase in misclassification of treatment outcome.

Serological detection of *B. gibsoni* antibodies is also a sensitive test for infection, but antibodies persist for several months after successful treatment making the test less useful for monitoring the efficacy of treatment. The pregnant dog in this study that produced infected puppies was negative by both PCR and serology during intake screening, a condition that could be explained by acute infection. Repeated PCR testing for several months after treatment is currently recommended to confirm treatment success in individual animals, which is often defined as 2 consecutive negative test results. However, it is recognized that treatment can suppress the parasite burden to below the limits of molecular detection rather than eliminate the infection entirely.[13] Dogs with clinical illness related to *B. gibsoni* infection often improve after treatment, even when parasitemia does not fully resolve.

Law enforcement agencies and humane organizations often work with limited resources when caring for

animals in large confiscations. At the time of this report, mass screening for *B. gibsoni* in dogfighting cases was still uncommon. Large dogfighting cases frequently require the establishment of temporary shelters staffed by rotating teams of emergency responders from national organizations. Under shelter conditions, expensive medication, long hold times, and repeated diagnostic testing can be logistically and cost-prohibitive. This creates an obstacle to screening and management of *B. gibsoni* infections in dogfighting cases.

Reducing treatment costs and duration of monitoring after treatment would make management of *B. gibsoni* accessible to more dogs rescued during dogfighting investigations. Use of compounded atovaquone in this study reduced drug costs by more than 50% without compromising apparent treatment efficacy. Caution should be exercised whenever compounded drugs are used, as it cannot be assumed that composition, concentration, bioavailability, and stability are consistent between different pharmacies or from batch to batch.

In this study, post-treatment monitoring results indicate there are several options for reducing dog holding times and minimizing testing costs without increasing the overall proportion of dogs with misclassified treatment outcome results. These options include moving post-treatment PCR testing to 30 and 60 days. This appears to achieve outcomes at the population level that are similar to those reported for more prolonged monitoring protocols. Dogs with a negative PCR test result at 30 days were highly likely (95%) to have negative results when retested at 60 days. This suggests that it might be unnecessary to hold dogs with a negative 30-day test for retesting at 60 days. Dogs with a positive PCR test result at 30 days should be held for retesting according to traditional testing intervals. Only 5 of the dogs in this study that were PCR-negative at 30 days were tested at 90 days, Therefore, it is not possible to compare the final outcomes with those of the previous treatment study.[15] The use of compounded medication and an abbreviated follow-up period might not be appropriate for individually owned dogs or dogs with clinical disease.

Footnotes

[a] MEPRON, GlaxoSmithKline, Research Triangle Park, NC
[b] Atovaquone Oral Suspension, Amneal Pharmaceuticals, Bridgewater, NJ
[c] Wedgewood Pharmacy, Swedesboro, NJ
[d] RealPCR, IDEXX Laboratories, Inc., Sacramento, CA

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Use of azithromycin and atovaquone for treatment of *Babesia gibsoni* in dogs is off-label in the United States.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Serological and PCR testing results in individual dogs for *Babesia gibsoni* after 10 days of 1 treatment with azithromycin and atovaquone.