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Re-emergence of *Babesia conradae* and effective treatment of infected dogs with atovaquone and azithromycin

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ABSTRACT

Babesia conradae (*B. conradae*) causes hemolytic anemia in dogs. This organism has not been reported clinically since it was originally described in southern California in 1991. To date, no anti-protozoal therapies have been associated with clearance of *B. conradae*. This report describes the use of atovaquone and azithromycin for the treatment of dogs naturally infected with *B. conradae* and report the re-emergence of *B. conradae* in southern California. Twelve dogs naturally infected with *B. conradae* were identified by practicing veterinarians and public health officials in southern California. Treatments consisted of a 10 day course of atovaquone (13.3 mg/kg PO q 8 h) and azithromycin (10–12.5 mg/kg PO q 24 h). Four dogs were treated in a randomized blinded placebo-controlled fashion, four additional cases were treated in a non-random, non-blinded fashion and one dog received no treatment. All dogs were tested for *B. conradae* DNA by polymerase chain reaction (PCR) initially and then once or 3 times post treatment (60–210 days). *B. conradae* infected dogs that received treatment did not have any detectable *Babesia* DNA by PCR after treatment. In contrast, dogs receiving placebo had detectable *Babesia* DNA by PCR throughout the study period. Combination therapy with atovaquone and azithromycin appears to be effective for acute and chronic babesiosis caused by *B. conradae*.

1. Introduction

Canine babesiosis is an important tick-transmitted protozoal disease that is characterized by hemolytic anemia and thrombocytopenia (Birkenheuer et al., 2005). Historically canine *Babesia* spp. have been classified as "large" or "small" based on the size of their intra-erythrocytic form. Recent molecular analyses have revealed at least three genetically distinct small *Babesia* parasites in the dog that include *Babesia gibsoni*, *Babesia/Theileria annae*, and *Babesia conradae*. Despite sharing similar phenotype these three parasites are genetically only distantly related (Criado-Fornelio et al., 2003; Kjemtrup et al., 2000).

B. conradae was discovered in 1990 when it was found to be the causative agent of hemolytic anemia in 11 dogs in southern California (Conrad et al., 1991). The original

Abbreviations: PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; rRNA, ribosomal ribonucleic acid; CA, California; LAC, Los Angeles County.

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description and several subsequent studies presumed the organism to be *B. gibsoni* based on microscopic appearance; however, further investigations determined that *B. conradae* is genetically and antigenically distinct from *B. gibsoni* (Kjemtrup and Conrad, 2006a; Kjemtrup et al., 2006b; Zahler et al., 2000a, 2000b). Compared to *B. gibsoni*, *B. conradae* seems to cause greater parasitemia, more profound anemia, higher mortality rates, and higher rates of disease recrudescence (Conrad et al., 1991; Meinkoth et al., 2002). In fact, five of eleven dogs in the original report died of babesiosis (Conrad et al., 1991).

The susceptibility of *B. conradae* to anti-babesial therapy has not been well characterized. In the original case series, treatment with diminazene aceturate or imidocarb dipropionate failed to successfully clear the infection (Conrad et al., 1991). Several other piroplasms including *B. gibsoni*, *B. microti* and *Cytauxzoon felis* have been shown to respond to combined atovaquone and azithromycin treatment (Krause et al., 2000; Birkenheuer et al., 2004; Cohn et al., 2011). The purpose of this study is to describe the use of combination therapy with azithromycin and atovaquone in dogs with *B. conradae* infections and to remind practitioners that *B. conradae* continues to cause canine babesiosis in southern California.

2. Materials and methods

2.1. Dogs

Twelve cases in which *B. conradae* infection was documented by PCR (North Carolina State University College of Veterinary Medicine Vector-Borne Disease Diagnostic Laboratory, Raleigh, North Carolina; ANTECH Diagnostics Inc., Irvine, California) and light microscopic examination of stained blood smears were identified by practicing veterinarians and public health officials in southern California. Eight dogs were of mixed breeding and had no history of travel outside of Los Angeles County (LAC), CA. These 8 dogs, including 3 intact males and 5 intact females, were related to each other and originated from a single household. All of these dogs were descendents of a single female (dog 9) (Fig. 1). Their ages ranged from 11 weeks to 6 years. At the time of the study, 5 of the 8 mixed breed dogs remained in the same household and 3 had been adopted into new homes at different locations. The remaining *B. conradae* cases were unrelated and include an 8 year old spayed female "Bull Terrier" type dog and three six-week-old female (breed unknown) littermates from a shelter in LAC.

Four of the 9 infected dogs were acutely ill, as characterized by severe lethargy and pallor, at the time of treatment. The clinically affected dog in the placebo group illness was transient resolving within 5 days of initial presentation. The remaining dogs were either considered normal by their caretaker or only displayed mild signs of illness such as lethargy at the time of treatment. The mean PCV from the 3 dogs in which this parameter was measured was 10% (range 8–16%; reference range 39–58%). Platelet counts were measured in two of these dogs and were 56×10^3 and 83×10^3 (reference range $190–468 \times 10^3$). The six-week-old puppies were anemic (data not available)

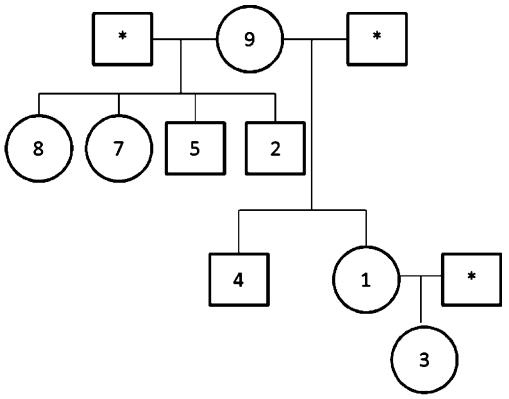

Fig. 1. Relationship of the 8 mixed breed *Babesia conradae* infected dogs. Legend: □, Male; ○, Female; *, Unknown *B. conradae* infection status. Numbers correspond to dog numbers in Tables 1 and 2.

and euthanized before any treatments were administered (Table 1).

2.2. Treatment protocol

With informed owner consent, 4 *B. conradae* cases that were clinically stable (i.e. did not require hospitalization or emergency care) and living in the same household were randomized and treated in a blinded placebo-controlled

Table 1
Clinical signs and clinicopathologic data of *B. conradae* cases at the time of treatment.

Dog	Treatment	Clinical signs at time of treatment	Clinicopathologic data
1	Atovaquone and Azithromycin[a]	None	N.A.
2	Atovaquone and Azithromycin[a]	Lethargic, pallor, vomiting	N.A.
3	Placebo	None	N.A.
4	Placebo	Lethargic, pallor	PCV 8%, Platelets 56 k
5	Atovaquone and Azithromycin[b]	Lethargic, pallor	PCV 8%
6	Atovaquone and Azithromycin[b]	Lethargic, pallor	PCV 16%, Platelets 83 k
7	Atovaquone and Azithromycin[b]	None	N.A.
8	Atovaquone and Azithromycin[b]	None	N.A.
9	No Treatment	None	N.A.
10	Euthanasia	N.A.	N.A.
11	Euthanasia	N.A	N.A.
12	Euthanasia	N.A.	N.A.

PCV reference range 39–58%; platelets reference range $190–468 \times 10^3$. N.A.: Not applicable.

[a] Randomized blinded placebo-controlled.

[b] Non-random, non-blinded.