

Emergence of *Babesia Conradae* Infection in Coyote-hunting Greyhounds in Oklahoma, USA

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Keywords: Babesia conradae, Babesiosis, Canine, Dog, Oklahoma, Greyhound, Anemia, Thrombocytopenia, 18S rRNA

Posted Date: January 28th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-154040/v1>

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Version of Record: A version of this preprint was published at Parasites & Vectors on August 14th, 2021. See the published version at <https://doi.org/10.1186/s13071-021-04897-x>.

Abstract

Background: *Babesia* species are intraerythrocytic Apicomplexan parasites that infect a wide range of vertebrate hosts. These pathogens are typically transmitted either by tick vectors or by direct blood-to-blood contact, and may cause life-threatening clinical disease such as thrombocytopenia, hemolytic anemia, and acute renal failure in canine hosts. While *Babesia vogeli* and *Babesia gibsoni* infections have both been reported in Oklahoma, reports of *B. conradae* infections have been limited to California.

Methods: Whole blood samples were collected in EDTA tubes from all dogs in four separate kennels in Oklahoma. DNA was extracted from each blood sample and a nested PCR was performed using general Apicomplexan primers for the partial 18S rRNA gene. PCR products were electrophoresed in agarose matrix and appropriately sized amplicons were sequenced. Sequences were compared to reference 18S rRNA sequences available in GenBank, and samples with >98% homology to *B. conradae* (GenBank MK256976) were considered positive. *B. conradae* positive dogs were then treated with atovaquone (13.5 mg/kg TID) and azithromycin (10 mg/kg SID) for 10 days and retested at 30 and 60 days post treatment by PCR.

Results: Fifteen of 40 dogs tested positive for *B. conradae* with 98–100% sequence homology to *B. conradae* from California. All positive cases were coyote-hunting Greyhounds. Treatment of clinically ill dogs with atovaquone and azithromycin resulted in complete clinical recovery in clinically ill dogs and all treated dogs had negative follow-up PCR at 30 and 60 days post treatment.

Conclusions: Collectively, this study (i) documents the occurrence of *B. conradae* in Oklahoma, (ii) highlights this pathogen as a differential to be considered when clinical signs are present, and (iii) supports the use of atovaquone and azithromycin as effective treatment in these cases.

Background

Babesia species are intraerythrocytic protozoan parasites in the phylum Apicomplexa that are transmitted by the bite of an infected tick or by passage of contaminated blood to a susceptible, naïve host. There are over 100 species described, which are divided into two broad categories: small *Babesia* (measuring 1–3 µm) and large *Babesia* (measuring 3–7 µm). There are currently five named *Babesia* spp. enzootic to the United States known to infect dogs: *Babesia vogeli* (large), *Babesia* sp. (Coco isolate, large), *B. gibsoni* (small), *B. vulpes* (formerly *Theileria annae*, small), and *B. conradae* (small) (1–4).

Babesia conradae was first reported in California in 1991 as *B. gibsoni*, given that *B. gibsoni* was the only small *Babesia* sp. known to infect dogs at the time (5). Further characterization of the piroplasm in 2006 revealed that the California organism was a distinct species and the name was changed to *B. conradae* after the first reporting author, Dr. Patricia Conrad (7). Transmission dynamics of *B. conradae* remain unknown as attempts at tick transmission of this piroplasm have not been successful (8).

Dogs infected with *B. conradae* exhibit typical clinical signs of babesiosis including anorexia, hemolytic anemia, splenomegaly, thrombocytopenia, and vomiting (5). Similar to other canine *Babesia* spp., clinical signs resulting from *B. conradae* infection can vary and range from mild to life-threatening. Severe complications such as acute renal failure/renal disease, cardiac related alterations, acute respiratory distress syndrome, and acute pancreatitis may result, particularly in dogs with *B. conradae* infection (2, 5). Membranoproliferative glomerulonephritis has been reported in one patient. Case fatality rate in *B. conradae* patients can reach up to 40% without timely and appropriate therapeutic intervention (5). While various medications have been attempted, combination therapy with atovaquone and azithromycin is the only treatment regimen shown to successfully clear *B. conradae* infection in dogs (9).

B. vogeli and *B. gibsoni* infections have been previously reported throughout the United States and in Oklahoma; however, *B. conradae* infection has not yet been documented outside of the state of California. In the current study, we describe four separate kennel outbreaks of *B. conradae* infection in Oklahoma. Dogs infected with *B. conradae* were then treated with atovaquone and azithromycin (9, 10), which resulted in complete resolution of clinical signs in the symptomatic dogs and negative PCR tests at 30- and 60- days post-treatment in all cases.

Methods

Aim

The aim of this study was to survey kennelled coyote-hunting dogs for *B. conradae* and other apicomplexan parasites and to establish efficacy of published atovaquone and azithromycin therapy in infected dogs.

Study Population

A total of 40 dogs from four separate kennels in Oklahoma were included in this study. Group 1 consisted of 6 Greyhounds and 4 Treeing Walker Coonhounds from Crescent, OK sampled in March of 2014; Group 2 consisted of 16 Greyhounds from Kingfisher, OK also sampled in March of 2014; Group 3 consisted of 10 Greyhounds from Vinita, OK sampled in February of 2019; and Group 4 consisted of 4 Greyhounds from Hobart,

OK sampled in May of 2020 (Figure 1). One dog from Group 1 displayed clinical signs of lethargy, fever, and anemia while the remaining dogs were subclinical. Clinical signs were not apparent in any of the dogs in Group 2. In Group 3, clinical signs including vomiting, lethargy, anorexia, and anemia were observed in 2 dogs, while the remaining dogs were subclinical. Group 4 had one clinical dog, which died prior to diagnosis and treatment (Dog 37) and one subclinical dog. At initial presentation, whole blood (1–5 mL) was collected in EDTA from all animals (n=40) and transported on ice overnight to the Oklahoma State University College of Veterinary Medicine (OSU-CVM). Samples were stored at 4°C prior to processing.

DNA Extraction and PCR

For Groups 1 and 2, DNA was extracted from whole blood using the DNeasy Blood and Tissue kit (QIAGEN, Valencia, CA). For Groups 3 and 4, DNA was extracted using the Illustra™ blood genomic Prep Mini Spin Kit (GE Healthcare, Piscataway, New Jersey). All extractions were performed according to kit manufacturer instructions. Dedicated laboratory areas were utilized for DNA extractions, primary and secondary PCR amplifications, and PCR product purifications to prevent contamination events. Separate ultra-purified water samples (NTC) were included as negative controls in DNA extractions and PCR amplifications. DNA extracts from Groups 1–4 were analyzed by previously described nested PCR methods which amplify a 460- to 520-bp hypervariable region of the 18S rRNA gene of *Babesia* spp. and some other apicomplexans (Table 1) (11, 12), except for Dog 27 whose PCR and sequencing was carried out by the North Carolina State Vector Borne Disease Diagnostics Lab (NCSVBDDL) per their standard operating procedures. NCSVBDDL utilizes primers that also amplify the 18S rRNA gene. DNA extracts from known piroplasm positive blood samples by microscopy were included as positive controls for each sample set.

Table 1. PCR primers used to amplify partial 18S rRNA gene of *Babesia* spp. and other apicomplexans.

Primer	Primer Sequence (5'→3')	Reference
BABA-F	CCG AAT TCG ACA ACC TGG TTG ATC CTG CCA GT	11
BABA-R	CCC GGA TCC AAG CTT GAT CCT TCT GCA GGT TCA CCT AC	11
3.1	CTC CTT CCT TTA AGT GAT AAG	12
5.1	CCT GGT TGA TCC TGC CAG TAG T	12
RLB-F	GAG GTA GTG ACA AGA AAT AAC AAT A	12
RLB-R	TCT TCG ATC CCC TAA CTT TC	12

For Groups 1 and 2, primary PCR reactions were performed in 25 µL volumes containing 0.25 U Taq polymerase (Promega, Madison, WI), 10× Taq buffer (Promega), 1.5 mM MgCl₂, 0.8 mM dNTP mixture (Promega), 0.8 µM each primer BABA-F and BABA-R, and 5 µL template DNA. Primary reaction conditions were as follows: 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 56.6°C for 1 min, 72°C for 2 min, and a final extension step of 72°C for 5 min. Nested PCR was carried out using 1 µL of the primary product and primers RLB-F and RLB-R. Nested reaction conditions were as follows: 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 2 min, and a final extension step of 72°C for 5 min.

For Groups 3 and 4, primary PCR reactions were prepared in 25 µL volumes containing 0.075 U Accuprime™ Taq HIFI (ThermoFisher, Waltham, MA), 1X AccuPrime™ PCR Buffer II (ThermoFisher), 1.5 mM MgSO₄ (ThermoFisher), 0.2 µM each primer 3.1 and 5.1, and 5 µL of DNA extract. Primary reaction conditions were as follows: 94°C for 2 min followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, 68°C for 1.5 min, and a final extension step of 72°C for 10 min. Nested PCR was again carried out using 1 µL of primary product and primers RLB-F and RLB-R, but cycling conditions were different than above. For Group 3 and 4 dogs, nested PCR reaction conditions were as follows: 94°C for 2 min followed by 40 cycles of 94°C for 1 minute, 50°C for 1 minute, 68°C for 1.5 minutes, and a final extension step of 72°C for 10 minutes.

PCR Product Purification and Sequencing

PCR products were electrophoresed in a 2% agarose matrix containing either GelRed® QIAquick® Gel Extraction Kit (Qiagen, Valencia, CA) or the Wizard® SV Gel and PCR Clean-Up System (Promega) according to manufacturer's instructions. DNA sequencing was performed at the Oklahoma State University Molecular Core Facility (Stillwater, OK) with an ABI 3730 DNA Analyzer, except for Dog 27. Forward and reverse sequences were aligned with ClustalW (Bioinformatics Center, Kyoto, Japan) and compared with sequence data available in the National Center for Biotechnology Information database (GenBank) for *B. conradae* (MK256976), *B. canis* (AY272047), and *B. gibsoni* (KC461261) to determine percent homology. Samples were considered positive if they were 98% or higher homologous to *B. conradae* (GenBank MK256976). The sequences from Dogs 1, 28, 29, 33, 34, 35, 37 and 38 have been deposited in GenBank under the accession numbers MW1470222, MT430944, MW145168, MW145196, MW145199, MW145504, MW145505 and MW145506, respectively.

Phylogenetic Tree and Percent Identity Matrix Construction

All partial 18S sequences obtained from canines in the current study were entered into MacVector, aligned and trimmed along with various 18S piroplasm reference sequences available in GenBank which were used in previous analyses (1, 7, 13). A maximum likelihood phylogenetic tree was constructed using unweighted pair group method with arithmetic mean (UPGMA) analysis. Bootstrap values are based on 1000 replicates and only bootstraps >50% are indicated. The percent homology matrix was also produced in MacVector using the same 18S full and partial sequences.

Treatment Protocol

Upon owner consent, surviving *B. conradae* PCR positive dogs from each cohort were treated with a previously described treatment regimen shown to eliminate infection *in vivo* (9). Atovaquone (GlaxoSmithKline, Research Triangle Park, NC) and azithromycin (Pfizer, New York, NY) was compounded in an oral suspension and administered to all surviving PCR-positive dogs at a dose of 13.5 mg/kg TID (atovaquone) and 10 mg/kg SID (azithromycin) for 10 days. Whole blood (1–5 mL) was collected in EDTA prior to treatment (day 0) and at 30 and 60 days post-treatment. DNA was extracted from each blood sample and tested by PCR to detect *Babesia* sp. infection as previously described.

Statistics

The prevalence of *B. conradae* infection was calculated according to Bush et al. (14) and 95% confidence intervals were calculated using QuickCalcs (15). The prevalence of *B. conradae* in hunting dogs among kennels was compared using chi-square tests (16).

Results

PCR of Dogs

From the 40 coyote hunting dogs screened for *B. conradae* infection, 15 (37.5%; 24.1%–53.0%) were positive by PCR and sequencing for *B. conradae* infection (Table 2). This included 3 of 10 (30.0%; 10.3%–60.8%) dogs from Group 1 (Crescent, OK; all Greyhounds), 4 of 16 (25%, 9.7%–50.0%) dogs from Group 2 (Kingfisher, OK; all Greyhounds), 6 of 10 (60.0%, 31.2%–83.3%) dogs from Group 3 (Vinita, OK; all Greyhounds), and 2 of 4 (50.0%; 15.0%–85.0%) dogs from Group 4 (Hobart, OK; all Greyhounds). A significant difference in the prevalence of *B. conradae* among the kennels was not detected ($X^2 = 3.733$, $df = 2$, $p = 0.292$).

Table 2. PCR results of coyote hunting dogs in Oklahoma tested for infection with *Babesia conradae*.

Dog number	Age in years	Gender	Breed	Group	Location	Day 0	Day 30	Day 60
1	4	NA	Greyhound	1	Crescent, OK	+	-	-
2	2	NA	Greyhound	1	Crescent, OK	-	NA	NA
3	4	NA	Greyhound	1	Crescent, OK	+	-	-
4	2	NA	Greyhound	1	Crescent, OK	+	-	-
5	3	NA	Greyhound	1	Crescent, OK	-	NA	NA
6	0.75	NA	Greyhound	1	Crescent, OK	-	NA	NA
7	6	NA	Treeing walker coonhound	1	Crescent, OK	-	NA	NA
8	5	NA	Treeing walker coonhound	1	Crescent, OK	-	NA	NA
9	4	NA	Treeing walker coonhound	1	Crescent, OK	-	NA	NA
10	0.75	NA	Treeing walker coonhound	1	Crescent, OK	-	NA	NA
11	1.5	NA	Greyhound	2	Kingfisher, OK	+	-	-
12	5	NA	Greyhound	2	Kingfisher, OK	-	NA	NA
13	NA	NA	Greyhound	2	Kingfisher, OK	+	-	-
14	6	NA	Greyhound	2	Kingfisher, OK	-	NA	NA
15	3	NA	Greyhound	2	Kingfisher, OK	-	NA	NA
16	1.6	NA	Greyhound	2	Kingfisher, OK	-	NA	NA
17	1.6	NA	Greyhound	2	Kingfisher, OK	-	NA	NA
18	0.4	NA	Greyhound	2	Kingfisher, OK	-	NA	NA
19	1.5	NA	Greyhound	2	Kingfisher, OK	+	-	-
20	1.5	NA	Greyhound	2	Kingfisher, OK	+	-	-
21	10	NA	Greyhound	2	Kingfisher, OK	-	NA	NA
22	0.4	NA	Greyhound	2	Kingfisher, OK	-	NA	NA
23	0.4	NA	Greyhound	2	Kingfisher, OK	-	NA	NA
24	7	NA	Greyhound	2	Kingfisher, OK	-	NA	NA
25	0.5	NA	Greyhound	2	Kingfisher, OK	-	NA	NA
26	7	NA	Greyhound	2	Kingfisher, OK	-	NA	NA
27	3	F	Greyhound	3	Vinita, OK	+	-	-
28	5	F	Greyhound	3	Vinita, OK	+	-	-
29	6	M	Greyhound	3	Vinita, OK	+	-	-
30	1	F	Greyhound	3	Vinita, OK	-	NA	NA
31	0.9	M	Greyhound	3	Vinita, OK	-	NA	NA
32	1	F	Greyhound	3	Vinita, OK	-	NA	NA
33	5	F	Greyhound	3	Vinita, OK	+	-	-
34	7	F	Greyhound	3	Vinita, OK	+	-	-
35	2	M	Greyhound	3	Vinita, OK	+	-	-
36	1	M	Greyhound	3	Vinita, OK	-	NA	NA
37	6	M	Greyhound	4	Hobart, OK	+	Deceased	Deceased
38	3	M	Greyhound	4	Hobart, OK	+	-	-
39	0.75	M	Greyhound	4	Hobart, OK	-	NA	NA

Dogs treated with A&A therapy are indicated in **BOLD** (see text for details).

PCR positive dogs ranged in age from 1.5–6 years (\bar{x} =3.7 years; 95% CI 2.6–4.8), with age unknown in one positive dog. PCR negative dogs ranged in age from 5 months to 10 years (\bar{x} =2.8 years; 95% CI 1.68–3.9). There was no significant difference in mean ages of PCR positive and negative dogs (P =0.817). Of the PCR positive dogs in Group 3, four were females and two were males. Of the positive dogs in Group 4, both were males.

Clinicopathologic Findings

Clinicopathologic data available for dogs in this study consisted of regenerative anemia, thrombocytopenia, hyperglycemia, hypocalcemia and in one case, severe azotemia. We were unable to obtain a sequence on the dog with severe azotemia and it is therefore not included in the total sample set. Postmortem examination of this case revealed marked membranoproliferative glomerulonephritis and tubular proteinosis, as well as myocardial necrosis and necrosuppurative opportunistic bronchopneumonia that cultured *Escherichia coli*, *Staphylococcus pseudintermedius* and *Streptococcus minor*. Blood smear evaluation of infected dogs from Group 1 revealed mild polychromasia (indicative of regenerative anemia) and numerous basophilic, intraerythrocytic piroplasms consistent with *Babesia* sp. (Figure 2).

Sequence Analysis

All PCR positive samples exhibited 98–100% homology to *B. conradae* (GenBank MK256976, AF158702) and 94–100% homology with each other, and shared 81–89% homology with *B. gibsoni* and *B. canis* (Table 3). Phylogenetic analysis demonstrated a distinct relationship between the Oklahoma *Babesia* sp. and the California *B. conradae* strains; sequences from Oklahoma dogs clustered with *B. conradae* sequences documented from California dogs (GenBank MK256976 and AF158702), and were more distant to other *Babesia* spp. sequences used in the comparison (Figure 3).

Table 3. Percent identity matrix of Oklahoma *B. conradae* 18S rRNA sequences compared to reference sequences in GenBank.

	Dog 1	Dog 27	Dog 28	Dog 29	Dog 33	Dog 34	Dog 35	Dog 37	Dog 38	<i>Babesia conradae</i> (MK256976)	<i>Babesia gibsoni</i> (KC461261)	<i>Babesia canis</i> (AY272047)
Dog 1	100	97	99	98	98	98	98	95	95	98	81	82
Dog 27	97	100	98	98	97	97	97	94	98	99	88	89
Dog 28	99	98	100	100	98	99	99	99	99	99	81	82
Dog 29	98	98	100	100	98	99	99	98	99	99	81	82
Dog 33	98	97	98	98	100	98	98	99	98	98	82	82
Dog 34	98	97	99	99	98	100	99	98	98	98	81	82
Dog 35	98	97	99	99	98	99	100	99	99	99	81	82
Dog 37	95	94	99	98	99	98	99	100	98	99	84	84
Dog 38	95	98	99	99	98	98	99	98	100	99	84	83
<i>Babesia conradae</i> (MK256976)	98	99	99	99	98	98	99	99	99	100	86	86
<i>Babesia gibsoni</i> (KC461261)	81	88	81	81	82	81	81	84	84	86	100	94
<i>Babesia canis</i> (AY272047)	82	89	82	82	82	82	82	84	83	86	94	100

Of the PCR-positive dogs in Groups 1-4, fourteen (n=14) dogs (Table 2) were administered a combination therapy of atovaquone and azithromycin for 10 days as previously described and then re-tested by PCR at 30 and 60 days post-treatment. In one case (Dog 27) three blood

transfusions were required to stabilize the patient during treatment. Dog 37 passed away shortly after blood was collected for testing and was therefore not in the treatment group. Complete resolution of clinical signs was observed in all clinical dogs by day 4 of treatment, and *B. conradae* DNA was not detected in the blood of any dog at 30 or 60 days post-treatment.

Discussion

Multiple outbreaks of *B. conradae* have been previously reported in dogs from southern California since 1991 (5, 9, 17, 18), but infection has not yet been reported outside this core, initial area. A single report of *B. gibsoni*-like parasite genetically similar to *B. conradae* was documented in Oklahoma in 2001 (GenBank AF205636) (19), but further BLAST analysis by the authors showed 100% alignment with numerous *B. gibsoni* sequences and significant genetic divergence from *B. conradae* (76.8% homologous to *B. conradae*, GenBank AF158702). Full travel and family histories are unavailable for all dogs in the current study; however, none of the dogs in the current study originated from or had been transported to California. To the authors' knowledge, this is the first published report of *B. conradae* infection outside of California and represents the emergence of an important pathogen in Oklahoma that is capable of causing significant disease in kennel dogs.

Some *Babesia* spp. can infect multiple vertebrate species, such as *B. microti* identified in both rodents and humans and *B. divergens* identified in both cattle and gerbils (21). Phylogenetic analyses showed *B. conradae* is closely related to piroplasms from humans, bighorn sheep and mule deer (1). Determining suitability of various hosts for *B. conradae*, particularly humans, is an important area needing further research, especially when route of transmission remains undetermined. A maximum likelihood phylogenetic tree (Fig. 3) constructed from samples with sequence data and other sequences available in GenBank demonstrates a close relationship of the *B. conradae* strains from Oklahoma with those from California. It also demonstrates a clear distinction from *B. gibsoni* (1, 13).

Diagnosis of babesiosis is typically based on clinical signs and/or observation of intraerythrocytic piroplasms on blood smear (Fig. 2). Ancillary testing (PCR and DNA sequencing) is required for accurate identification given that small piroplasms are microscopically indistinguishable from each other both within the *Babesia* genus and from *Theileria* sp., and there is cross reactivity with immunofluorescence antibody testing (IFA) (20). Additionally, *B. conradae* parasitemia can be low (< 1% of erythrocytes with piroplasms), making detection of the organism on blood smear difficult. PCR has become widely available and is both sensitive and specific for detection of *B. conradae* DNA using the 18S rRNA gene (21). Differentiation between the small *Babesia* spp. is important, given that *B. conradae* can be more pathogenic than *B. gibsoni* (5, 9, 22).

Testing and disease identification have previously and primarily been in clinically ill dogs. However, in the current study, we observed 4 *B. conradae* positive dogs with clinical babesiosis and 10 with subclinical infections. Our observation of 10 subclinical dogs suggests some dogs may be chronic carriers that could serve as a reservoir of infection for naïve animals. Further research should determine if *B. conradae* is more prevalent in high risk groups than previously thought. It remains to be determined if there are any negative long-term health problems associated with a chronic carrier state and whether atovaquone and azithromycin treatment would be beneficial in these animals.

Babesia spp. are primarily transmitted by ticks worldwide; however, transmission mechanisms of *Babesia* spp. in the United States differ. For example, *B. vogeli* is transmitted by *Rhipicephalus sanguineus* sensu lato (brown dog tick), while *B. gibsoni* is largely considered to be transmitted by passage of contaminated blood during dog fights (5). Comparatively, the source of *B. conradae* infection in domestic dogs is still unknown, and it is unclear whether transmission occurs via tick a vector, by transfer of contaminated blood between dogs, trans-placentally from an infected dam, or by a previously undocumented route (8).

There is some evidence that other *Babesia* sp. can be transmitted trans-placentally, but a detailed family history was not available to assess the possibility for vertical *B. conradae* infection in these Greyhounds (23). Kennel owners in the present study suspected that their dogs were becoming infected with *B. conradae* by fighting coyotes while hunting. A previous report of *B. conradae* infection in coyote hunting dogs in southern California also documented that infection was associated with a history of aggressive interactions with coyotes (17). A serosurvey performed in California in 1994 showed that 3 of 9 coyotes were seropositive for *Babesia gibsoni*, suggesting these wild canids may be a reservoir host for *Babesia* sp. (18). At the time *B. conradae* had not yet been recognized, highlighting the potential for these seropositive coyotes to have been actually infected with multiple *Babesia* spp., including *B. conradae*. Moreover, these studies used IFA for organism detection, which is also subject to cross-reactivity (20). It is also possible to have a subclinical carrier dog transmit the disease to a naïve dog during hunting, as the dogs may bite each other during the interaction with the coyote. Although speculative at this time, all of these scenarios represent possible reservoirs for *B. conradae* infection in these study animals, and studies are currently underway to investigate the presence of *B. conradae* in wild tick populations, free-ranging coyotes, and other domestic canids via contact tracing to determine the source of this pathogen in the Oklahoma region.

In conclusion, the current study documents the emergence of *B. conradae* infection in 15 coyote-hunting Greyhounds in Oklahoma. *Babesia conradae* infection resulted in a variety of clinical signs including fever, vomiting, anorexia, regenerative anemia, and thrombocytopenia - all of which were mitigated by treatment with atovaquone and azithromycin. The source of *B. conradae* infection in Oklahoma remains unknown. At this time, *B. conradae* infection in Oklahoma has been documented in only Greyhounds used for hunting coyotes. Further research is needed to

determine the regional prevalence, reservoir host(s), mode(s) of transmission, and diversity of vertebrate host species. Similarly, surveillance should be expanded to include other areas of the Midwest and Southern United States.

Declarations

Ethics Approval and Consent to Participate

All samples were ethically collected from client-owned animals with owner informed consent that the samples will be used in research.

Consent for Publication

All samples and results were obtained, tested and prepared for publication with owner consent.

Availability of Data and Materials

The sequences from Dogs 1, 28, 29, 33, 34, 35, 37 and 38 have been deposited in GenBank under the accession numbers MW1470222, MT430944, MW145168, MW145196, MW145199, MW145504, MW145505 and MW145506, respectively.

Competing Interests

The authors declare that they have no competing interests.

Funding

Funding to cover diagnostics and treatment costs in these cases were provided by IDEXX, Pfizer and internal grants through the College of Veterinary Medicine, Oklahoma State University.

Author's Contributions

Authors MR and JT performed the sample collection, PCR and sequence analysis for Groups 1 and 2. GY was the primary clinician involved in sample collection and treatment administration for Group 1 while TB was the clinician for Group 3. ML performed DNA extraction, PCR and sequence analysis for Groups 3 and 4. RC, KA, CM and MR provided consultation and sample coordination while ES drafted the manuscript. All authors collaborated on manuscript assembly revision and submission.

Acknowledgments

We would like to thank the NC State Vector Borne Disease Diagnostics Lab for their cooperation and sequence contribution, Dr. Jim Meinkoth, DVM, PhD, DACVP for Figure 2, as well as Dr. Kathryn Duncan, DVM, for help constructing the Phylogenetic tree in MacVector.

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Figures

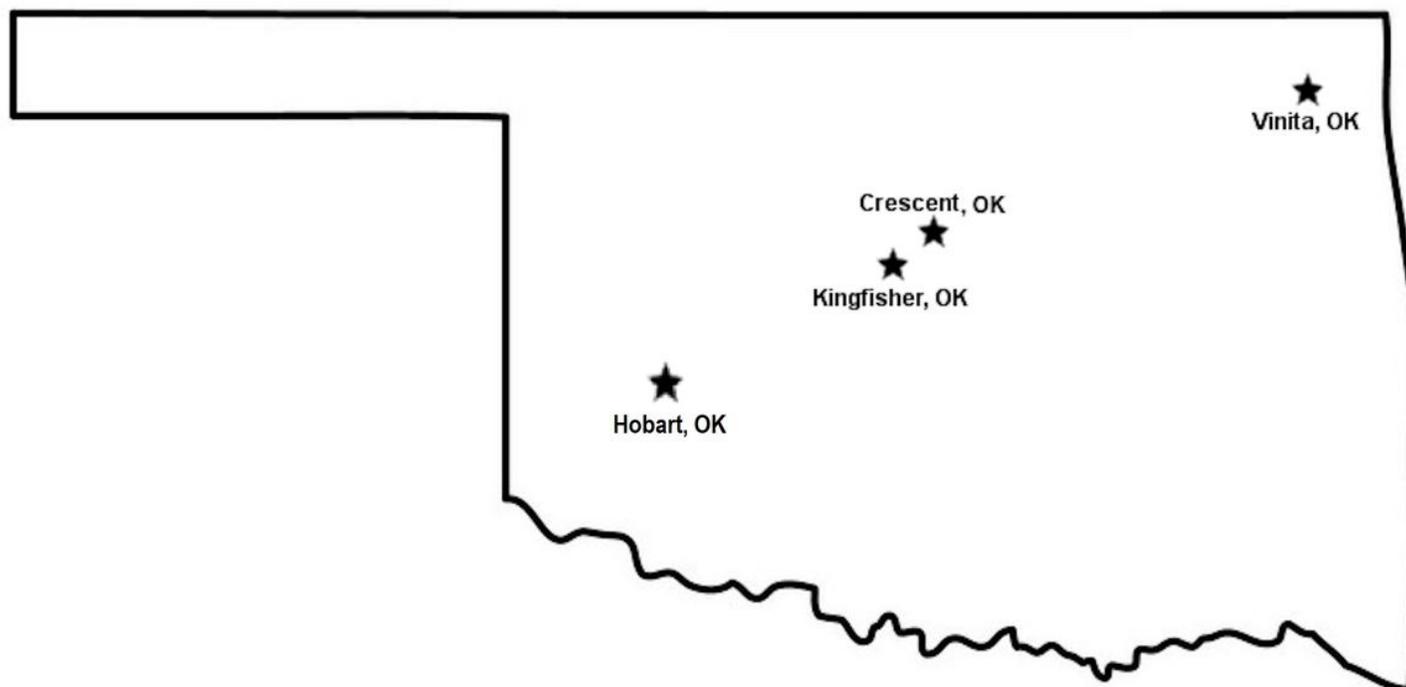


Figure 1

Geographical distribution of *B. conradae* cases in Oklahoma. A total of 40 dogs were included in this study from four separate kennels in Oklahoma; 6 Greyhounds and 4 Treeing Walker Coonhounds from Crescent, OK (Group 1), 16 Greyhounds from Kingfisher, OK (Group 2), 10 Greyhounds from Vinita, OK (Group 3), and 4 Greyhounds from Hobart, OK (Group 4).

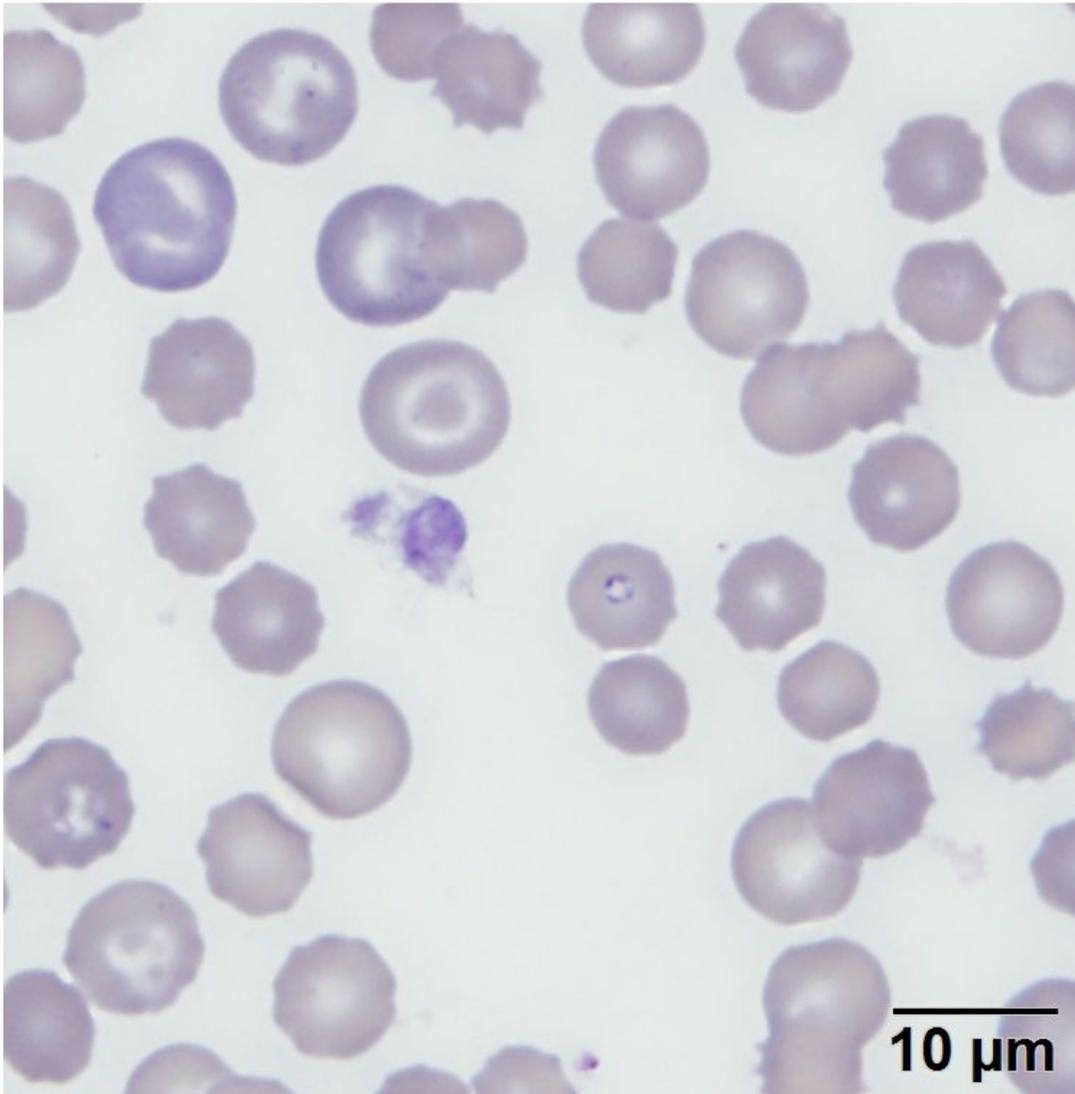


Figure 2

Blood smear from a *B. conradae* positive Greyhound in Group 1. Approximately 10% of the erythrocytes contain pale basophilic intracellular protozoa consistent with intraerythrocytic *Babesia conradae* piroplasms. A mild degree of polychromasia is also present within the specimen, indicating regenerative anemia (Scale bar = 10μm).

Method: UPGMA; Bootstrap (1000 reps); tie breaking = Random
 Distance: Uncorrected ("p")
 Gaps distributed proportionally

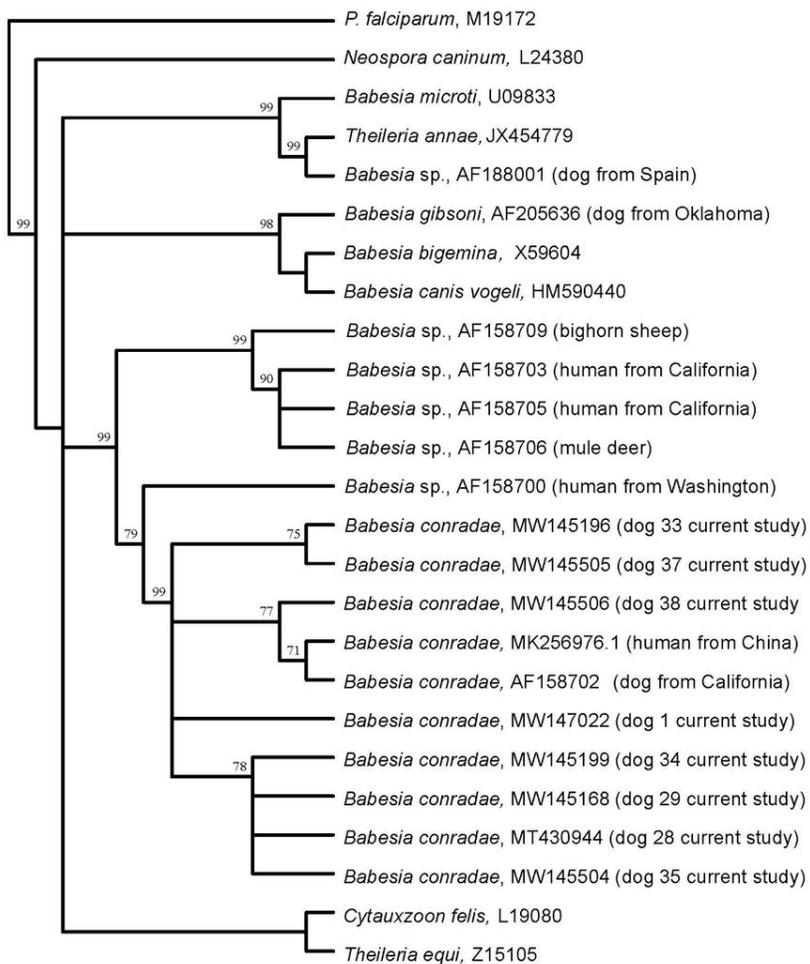


Figure 3

Phylogenetic comparison of *Babesia conradae* isolates from Oklahoma to other piroplasms. Maximum likelihood phylogenetic tree constructed using unweighted pair group method with arithmetic mean (UPGMA) analysis of 18S rRNA gene sequences extracted from GenBank. Clustering of study samples within a distinct subclade (red lines) illustrates the homology of these Oklahoma strains to the original (California) *Babesia conradae* strains (MK256976 and AF158702), and highlights the distinct separation of study samples from other *Babesia* spp. Bootstrap values are based on 1000 replicates and only bootstraps <50% are indicated.

Supplementary Files

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