Tick-Borne Relapsing Fever in Dogs

J. Piccione, G.J. Levine, C.A. Duff, G.M. Kuhlman, K.D. Scott, and M.D. Esteve-Gassent

Background: In the United States, Tick-Borne Relapsing Fever (TBRF) in dogs is caused by the spirochete bacteria *Borrelia turicatae* and *Borrelia hermsii*, transmitted by *Ornithodoros spp.* ticks. The hallmark diagnostic feature of this infection is the visualization of numerous spirochetes during standard blood smear examination. Although the course of spirochetemia has not been fully characterized in dogs, in humans infected with TBRF the episodes of spirochetemia and fever are intermittent.

Objectives: To describe TBRF in dogs by providing additional case reports and reviewing the disease in veterinary and human medicine.

Animals: Five cases of privately-owned dogs naturally infected with TBRF in Texas are reviewed.

Methods: Case series and literature review.

Results: All dogs were examined because of lethargy, inappetence, and pyrexia. Two dogs also had signs of neurologic disease. All dogs had thrombocytopenia and spirochetemia. All cases were administered tetracyclines orally. Platelet numbers improved and spirochetemia and pyrexia resolved in 4 out of 5 dogs, where follow-up information was available.

Conclusion and Clinical Importance: TBRF is likely underdiagnosed in veterinary medicine. In areas endemic to *Ornithodoros* spp. ticks, TBRF should be considered in dogs with thrombocytopenia. Examination of standard blood smears can provide a rapid and specific diagnosis of TBRF when spirochetes are observed.

Key words: Bacteremia; *Borrelia*; Spirochete; Spirochetemia; Thrombocytopenia.

Tick-Borne Relapsing Fever (TBRF) is caused by several bacteria in the genus *Borrelia*, excluding the causative agent of Lyme disease (*Borrelia burgdorferi*). TBRF is spread by feeding of *Ornithodoros* spp. ticks, which often goes unnoticed and which can transmit the *Borrelia* bacteria in seconds.[1] Clinical findings include pyrexia and possible lethargy, anorexia, and signs of neurologic disease. The hallmark feature of this infection is the visualization of numerous spirochetes (spirochetemia) during standard blood smear examination.[2] While CBC data can vary between dogs, all cases of TBRF are associated with severe thrombocytopenia. TBRF is likely underdiagnosed in veterinary medicine and could be an important consideration for dogs with thrombocytopenia in several areas of the United States.

Case 1

A 7-year-old female spayed Dachshund weighing 4.9 kg (10.8 lb) was referred to the Texas A&M

From the Texas A&M Veterinary Medical Diagnostic Laboratory, (Piccione); the Department of Veterinary Pathobiology, (Levine, Esteve-Gassent); and the Department of Small Animal Clinical Sciences (Duff, Kuhlman, Scott), College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX (Duff, Kuhlman, Scott).

Abbreviations:

CBC	complete blood count
IFA	immunofluorescence assay
RI	reference intervals
TBRF	tick-borne relapsing fever

University Veterinary Medical Teaching Hospital (TAMU VMTH) because of an increased rectal temperature, lethargy, and abnormal posture (tail tucking) for approximately 3 days. Examination revealed, mild mydriasis, prolonged pupillary light reflexes, exaggerated bilateral menace responses, and pyrexia (40.3°C [104.5°F]). The remainder of the physical exam revealed no abnormalities, including no evidence or clinical history of external parasites.

Plasma biochemistry revealed mild hypoalbuminemia (2.2 g/dL; RI: 2.4–3.6 g/dL). Abnormalities were not detected on routine urinalysis. Complete blood count revealed only a marked thrombocytopenia (47,000/μL; RI: 200,000–500,000/μL). However, blood smear examination revealed numerous spirochete bacteria (Fig. 1).

Antibodies to *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma spp.*, and *Dirofilaria immitis* antigen were not detected using an in-house enzyme-linked immunosorbent assay.[a] Leptospira DNA was not detected in urine by PCR.[b] Blood samples were sent to Rocky Mountain Laboratories[c] for IFA, amplification within mice, culture, and PCR for *Borrelia* spp. Rocky Mountain Laboratories performed PCR using primers that target *16SrRNA*, *flaB*, *gyrB*, and *glpQ* genes.[3]

Conventional PCR for the detection of Relapsing Fever *Borrelia* spp. was performed at Texas A&M University in an author's (MDEG) research laboratory using primers targeting the flagellin gene (*flaB*), *16SrRNA*, and *glpQ* genes.[4–6] The PCR assays targeting flagellin (*flaB*) and 16SrRNA genes are highly sensitive, whereas the PCR targeting *glpQ* specifically amplifies
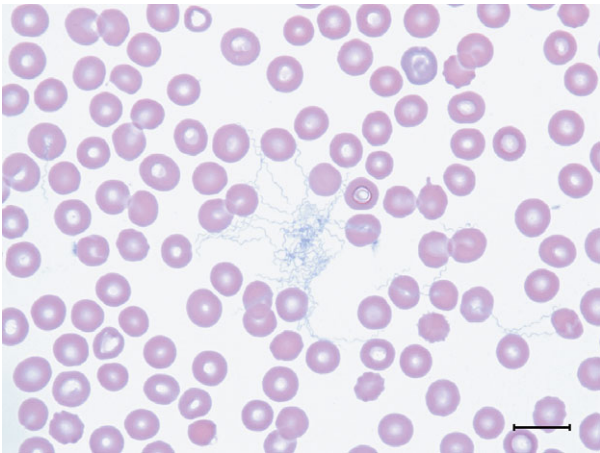
Fig 1. Peripheral blood. Numerous spirochete bacteria in a 7-year-old female spayed Dachshund (case 1). Modified Wright's; bar = 10 μm.

only relapsing fever species.[3–7] DNA was extracted from the buffy coat according to manufacturer's recommendations.[d] The DNA extraction and PCR amplification were carried out in separate laboratories and all PCR reactions were set up in a PCR cabinet.[e] In addition, a reagent negative control and a positive control containing *Borrelia burgdorferi* B31 MSK5 DNA were included in each reaction. At the time, a TBRF positive control was not available and sequencing was to be performed. PCR amplification was visualized by electrophoresis using 0.8% agarose gels and imaged using a ChemiDoc Touch.[™f] Amplification bands were cleaned and submitted for sequencing using both forward and reverse primers.[g] Chromatographs obtained through Eton Biosciences[g] were evaluated with the MacVector® *Assembler*[h] and a consensus sequence was generated for use in alignments, phylogenetic trees, and for construction of the identity matrix.

Utilizing the monoclonal antibody H9724 against the flagellin protein present in all species of *Borrelia*, IFA revealed the spirochetes were from the genus *Borrelia* (T. Schwan, personal communication). Analysis of sequences obtained from the PCR reactions performed at both laboratories confirmed that the infecting species was *Borrelia turicatae*. The 16SrRNA sequence obtained from the TAMU laboratory was published in GenBank® (accession number KP861623). The 490 bp fragment amplified corresponds with coordinates 445073 to 445562 on the *Borrelia turicatae* chromosome. This fragment is 100%, 99.8%, 97.6% identical to *B. turicatae* (U42299), *B. parkeri* (NR121776) and *B. hermsii* (M60968), respectively. In contrast, the amplified sequence was 34.6% identical to *B. burgdorferi* sensu stricto strain B31 (NC001318). All analysis were done in MacVector® *Assembler* 14.0[h] (Fig. 2A).

The dog was treated with intravenous crystalloid fluids (normosol-R) and doxycycline[i] (6 mg/kg [2.7 mg/lb] PO, q12h). Twenty-four hours after treatment was initiated, the dog was afebrile and no spirochetes were observed on blood smear examination. Administration of crystalloid fluids was discontinued, and the dog was discharged with instructions for a 28 day course of doxycycline[i] and re-examination with the referring veterinarian in 4 weeks.

Case 2

A 14-year-old female spayed Siberian Husky weighing 24.3 kg (53.5 lb) was examined at a private veterinary hospital in Waco, Texas for 3–4 days of inappetence and abnormal ambulation, characterized by ataxia and weakness. The dog was febrile (39.7°C [103.5°F]) and dehydrated at initial presentation. The dog had no recent clinical history of external parasites. In-house CBC data revealed lymphopenia (700/μL; RI: 1,000–4,800/μL) and severe thrombocytopenia (none detected /μL; RI: 200,000–500,000/μL), which was confirmed by blood smear examination. In addition, numerous spirochetes were observed throughout the smear. Serum biochemistry revealed mild increase in alkaline phosphatase activity (500 U/L (RI: 20–150 U/L). This abnormality had been repeatedly observed over 4 years prior to presentation.

Spirochetemia, marked thrombocytopenia (60,000/μL; RI: 200,000–500,000/μL) and mild lymphopenia (630/μL; RI: 1,000–4,800/μL) were confirmed at a diagnostic laboratory.[b] Initial diagnoses based on blood smear examination included nonpathogenic spirochetes and *Borrelia burgdorferi*. Indirect fluorescent antibody serology was positive for RMSF (sample screened at ≥1 : 16), but negative for *Ehrlichia canis* (CDC/V241 strain[j]) and Lyme borreliosis (B31 strain[j]).

Borrelia spp. conventional PCR was later performed at the diagnostic laboratory[b] using standard methods and following certified veterinary diagnostic laboratory approved standard operational procedures for molecular diagnostics. A negative reagent control was used; however, positive controls were unavailable. Direct, forward and reverse sequencing of the 16SrRNA product identified the spirochetes as *Borrelia turicatae* (GenBank® accession number KP861624). The 716 bp fragment amplified corresponds with coordinates 445350 to 446065 on the *Borrelia turicatae* chromosome. This fragment was 99.9% identical to *B. turicatae* (U42299) and *B. parkeri* (NR121776), and 97.3% identical to *B. hermsii* (M60968). In contrast, the amplified sequence was 31% identical to *B. burgdorferi* sensu stricto strain B31 (NC001318). All analysis were done in MacVector® *Assembler* 14.0[h]. These results were consistent with the dog being infected with the RF *Borrelia*, *B. turicatae* (Fig. 2B).

The dog was treated with doxycycline[i] (4 mg/kg [1.8 mg/lb]) and amoxicillin[k] (11 mg/kg [5 mg/lb]) orally twice daily for 28 and 14 days, respectively. A repeat CBC with blood smear examination 10 days later revealed mild thrombocytosis (592,000/μL; RI: 200,000–500,000/μL) and no visible spirochetes. The dog recovered uneventfully; however, hind limb weakness and pain persisted months after initial treatment. The dog was euthanized 6 months after initial presentation for
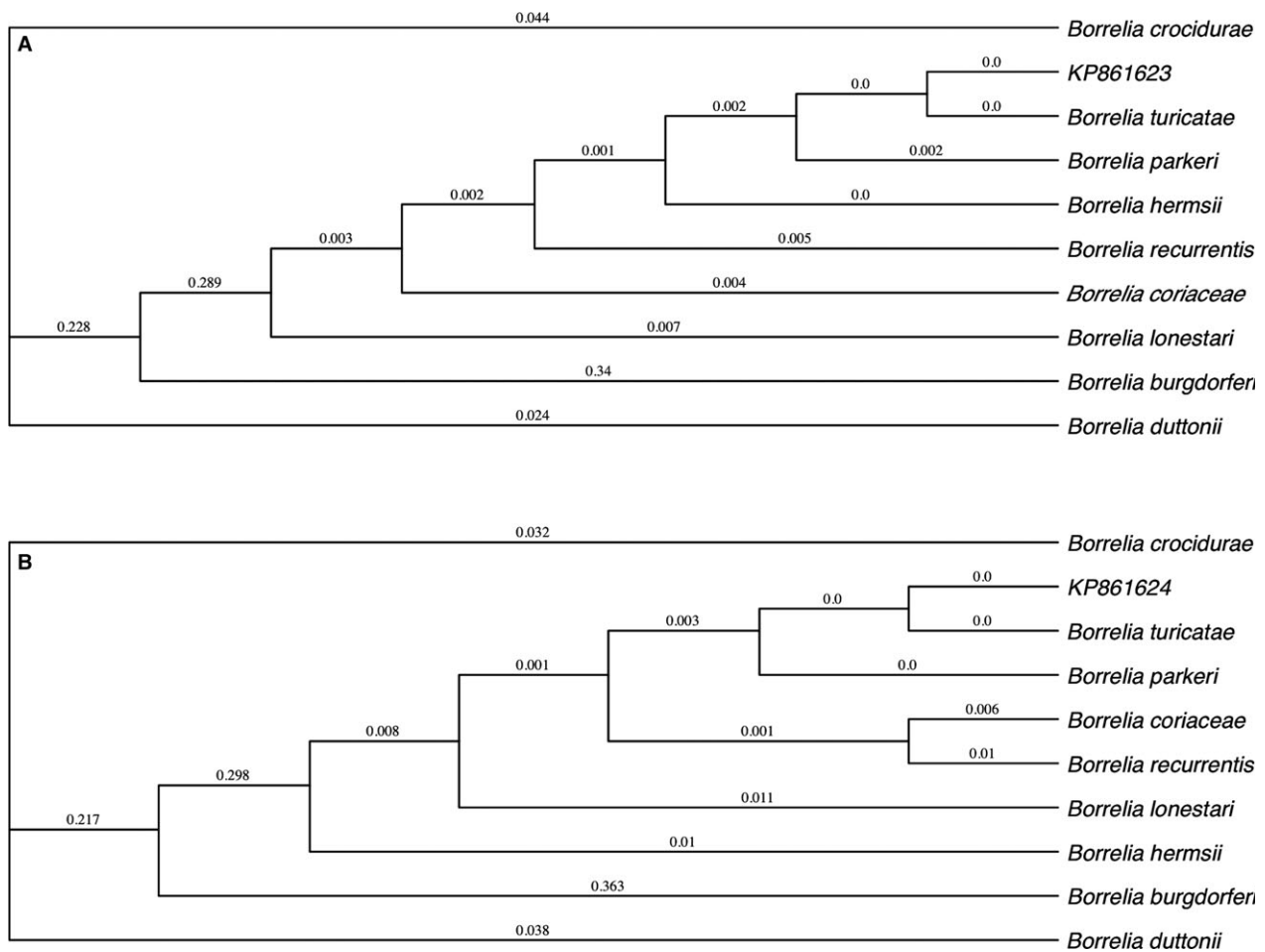
Fig 2. *Borrelia turicatae* strains detected in cases 1 (KP861623) (**A**) and 2 (KP861624) (**B**). The phylogenetic trees were generated utilizing the Neighbor joint method of aligned 16SrRNA sequences obtained from infected dogs, and representative species of the Relapsing Fever *Borrelia* group: *B. burgdorferi* (NC001314), *B. coriaceae* (U42286), *B. crocidurae* (KF176335), *B. duttonii* (CP000976), *B. hermsii* (M60968), *B. parkeri* (NR121776), *B. turicatae* (U42299), *B. lonestari* (AY166715) and *B. recurrentis* (AF107361). The phylogenetic tree was generated using MacVector® *Assembler* 14.0 (MacVector Inc.).

cognitive dysfunction and continued lumbosacral pain. A postmortem examination was not performed.

Case 3

A 10-year-old, spayed female mixed breed dog weighing 30 kg (66.4 lb) was examined at a private veterinary hospital in Smithville, Texas for a 1-week history of inappetence, lethargy and polydipsia. The dog was moderately febrile (40.2°C [104.4°F]). No external parasites were found on physical exam and no history of parasites was noted. In-house CBC data revealed a neutrophilia (23,200/µL; RI: 3,300–12,000/µL) and marked thrombocytopenia (44,000/µL; RI: 175,000–500,000/µL). In-house chemistry findings identified no relevant abnormalities. The dog was referred to the TAMU VMTH for further evaluation.

On presentation to TAMU VMTH the dog was lethargic, mildly dehydrated, and reluctant to rise and walk, with mild right stifle effusion present. There was a moderate leukocytosis present because of a neutrophilia with evidence of toxic change (25,852/µL; RI; 3,000–

11,500/µL). The dog was thrombocytopenic (45,000/µL; RI: 200,000–500,000/µL) and numerous extracellular spirochete bacteria were observed on blood smear examination. Abnormalities were not detected on a plasma chemistry panel. Antibodies to *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma spp.*, and *Dirofilaria immitis* antigen were not detected using an in-house enzyme-linked immunosorbent assay.[a] Doxycycline[i] antibiotic treatment was initiated (5 mg/kg [2.3 mg/lb] PO, q12h).

The next day the dog's temperature was 102.3°F (39.1°C), her attitude was mildly improved, and she was more willing to stand and walk. There was neutrophilia (21,344/µL; RI: 3000–11,500/µL) and thrombocytopenia (46,000/µL; RI: 200,000–500,000/µL). No spirochete bacteria were identified on blood smear examination. A 21-day course of doxycycline[i] (5 mg/kg [2.3 mg/lb] PO, q12h) was prescribed. An in-house CBC at the original private veterinary hospital was performed thirteen days after discharge, revealing no abnormalities. The dog was reported to be back to pre-illness mobility, activity level, and appetite.

PCR for *Borrelia* spp. was performed on the original sample at Texas A&M in an author's (MDEG) research laboratory. PCR was performed using the same conditions as those mentioned above (case one) except that case one's PCR product was used as a positive control for this case. A negative reagent control was utilized and no evidence of contamination was observed. All PCR reactions gave amplicons consistent with the *Borrelia turicatae* controls. Given the positive PCR results, clinical signs, and presence of spirochetemia, sequencing was not performed.

Case 4

An 11-year-old, intact male, Brittany Spaniel Mix weighing 26.6 kg (58.6 lb) was presented to a private veterinary hospital in Waco, Texas with a 2-week history of inappetence and lethargy. The dog was mildly febrile (39.6°C [103.2°F]) and had mild nasal discharge, which was chronic according to the owner. No external parasites were noted on physical exam or in the dog's history. There was a marked neutrophilia (50,950/μL; RI: 3,000–12,000/μL) and thrombocytopenia (5,000/μL; RI: 200,000–500,000/μL), which was confirmed by blood smear examination. In addition, numerous spirochetes were observed throughout the blood smear. In-house chemistry findings included a moderate increase in ALKP activity (700 U/L; RI: 20–150 U/L), and moderate to marked hypoalbuminemia (1.6 g/dL; RI: 2.5–4.4 g/dL).

PCR and sequencing were not performed in this case because the veterinarian was familiar with the diagnosis of Tick-Borne Relapsing Fever from a previous case (case 2). Indirect fluorescent antibody serology[b] was positive for *R. rickettsii* (sample screened at ≥1 : 16), and Lyme borreliosis (B31 strain[j], sample screened at ≥1 : 60). The dog was administered minocycline[l] (3.5 mg/kg [1.6 mg/lb] PO, q12h) for 28 days, and carprofen[m] (2 mg/kg [0.9 mg/lb] PO, q24 h) for fever and inflammation. The dog recovered uneventfully; however, died 8 months later secondary to a hemoabdomen from a presumed liver mass. The decreased albumin and increase in ALKP activity were attributed to liver disease and hemorrhage; however, vector-borne disease might have contributed to these abnormalities.

Case 5

A 10-year-old, female spayed, mixed breed dog weighing 29.1 kg (64.1 lb) was examined at a private veterinary hospital in Horseshoe Bay, Texas for a 3-day history of inappetence and lethargy. The dog was moderately febrile (40.3°C [104.6°F]) and had formed but mildly mucoid feces. No external parasites were noted on physical exam or in the dog's history. There was lymphopenia (430/μL; RI: 1,000–4,800/μL) and platelets were not detectable. Only low numbers of platelets were seen on blood smear examination. In addition, numerous spirochetes were observed throughout the smear. All analytes on an in-house chemistry analyzer were within reference intervals.

Visible spirochetemia and thrombocytopenia (17,200/μL; RI: 200,000–500,000/μL) were confirmed on standard blood smear examination at a diagnostic laboratory.[b] In addition, indirect fluorescent antibody serological testing for *Ehrlichia canis* (CDC/V241 strain[j], sample screened at ≥1 : 20), *R. rickettsii* (sample screened at ≥1 : 16), and Lyme borreliosis (B31 strain[j], sample screened at ≥1 : 60), was performed, with positive IFA results for each disease. PCR sequencing was not performed. The dog was administered doxycycline[i] (7.5 mg/kg [3.4 mg/lb] PO, q12h) for 6 weeks. The dog recovered uneventfully. Six weeks after initial diagnosis, CBC data revealed no abnormalities.

Discussion

The phylum Spirochaetes contains both pathologic and nonpathologic, gram-negative bacteria characterized by a coiled or spiral appearance. Spirochetes are responsible for several important veterinary diseases, including, but not limited to, leptospirosis, *Brachyspira* spp. infections, and Lyme disease.[8] While diseases caused by spirochetes are routinely suspected by veterinarians, visible spirochetemia has rarely been described. When molecular diagnostics are pursued, only tick-borne relapsing fever (TBRF) organisms have been found to cause spirochetemia detectable on standard blood smear examination. Original case reports, before advanced molecular diagnostics were available, mistakenly identified the spirochetes as *Borrelia burgdorferi* sensu stricto, the causative agent of Lyme disease.[9,10] *Borrelia burgdorferi* sensu stricto does not cause spirochetemia that is detectable on standard blood smear examinations.[11]

TBRF is associated with infection by a limited number of *Borrelia* spp., excluding *Borrelia burgdorferi* sensu stricto, and *B. recurrentis*, which causes [African] Relapsing Fever and is transmitted by lice.[12] In the United States, human cases of TBRF are mainly caused by three *Borrelia* species, including *Borrelia hermsii*, *Borrelia turicatae*, and *Borrelia parkerii*.

The *Borrelia* organisms of TBRF are transmitted by the bite of *Ornithodoros* species of soft ticks, which are located throughout the mid and southern United States.[13] Soft ticks feed for short duration (minutes) and are nocturnal, thus limiting the detection of these parasites.[12] In addition, some *Ornithodoros* species contain *Borrelia* organisms throughout multiple tissues concurrently (including the mid gut and salivary gland) which shortens organism transmission time during tick feeding. Transmission of TBRF *Borrelia* spp. can occur in as little as 15 seconds.[1] This is in sharp contrast with *Borrelia burgdorferi* sensu stricto which has to migrate from the mid gut to the salivary gland of its tick vector, *Ixodes scapularis*, to the host during feeding, requiring up to 18–24 hours for transmission.[12,14] In contrast with other tick-borne diseases, many animals can serve as reservoir and end hosts in TBRF.

TBRF is likely under recognized and underreported, limiting epidemiologic information.[2] In the Northwest United States, *Borrelia hermsii*, spread by *Ornithodoros*

hermsii ticks endemic to the area, is the most reported species of TBRF causing clinical cases in humans. In this area of the United States, infection has been most commonly associated with cabins. Rodents are the main reservoir hosts of *O. hermsii* ticks in this area.[2] In Texas, human cases of disease are caused by injection with *Borrelia turicatae* spread by *Ornithodoros turicatae* ticks in central Texas.[12] It is thought that TBRF exposure in Texas occurs mostly in caves and that many animals serve as hosts to *O. turicatae* ticks.[2] It is unclear if exposure to cabins and caves are the main sources of infections in animals because of the limited number of veterinary case reports. Thus far, the epidemiologic distribution of TBRF species in veterinary cases appears to be similar to that seen in human medicine. However, regions not previously thought to harbor these organisms, such as Florida, are being discovered through veterinary cases.[15,16]

In human cases of TBRF, the spirochetemia and concurrent episodes of fever are cyclic, with clinical signs lasting a few days and approximately 1 week elapsing between episodes.[2] Through the use of animal models, it has been suggested that antigenic variation allows spirochetes to evade the immune system.[17] For example, in mice, antigenic variation of the Vmps (variable major proteins) on the outer surface of the spirochete allows increased time in circulation, thus perpetuating clinical signs and tick acquisition rates.[17] In humans, TBRF is an acute disease, which can be effectively treated with antibiotics and supportive care; however, instances of reactivated infections have been reported in research animals and could be a concern in human and veterinary medicine.[18]

The earliest confirmed cases of TBRF in veterinary species were reported in the 1990's, with the first suspected cases seen decades earlier.[9,16] Reported cases of natural TBRF in animals in the last twenty years include rare case reports in dogs in Texas, Florida and Washington, a bat in the United Kingdom, and an aborted horse fetus from California.[15,16,19–21] Detectable spirochetemia on blood smear examination was only reported in dogs. It was unclear if blood smears were reviewed in the other cases. *Borrelia turicatae* was the predominant cause of TBRF in dogs, the same species identified in the dogs of this report where molecular diagnostics were performed (cases 1–3). The majority of dogs were from Texas, where *Borrelia turicatae* is the most common species to infect people. Recently, a dog from Washington was infected with *Borrelia hermsii*.[21] Clinical signs shared by the majority of dogs included: fever, ambulation or postural defects (arched back, lameness), anorexia/weight loss, and ocular lesions (uveitis, photophobia, corneal edema).[15,16,21]

The cases described in this report provide further information on the clinical presentation and laboratory findings of dogs infected with TBRF. All cases presented between May to August with vague clinical signs, such as lethargy, inappetance or both. The majority of dogs diagnosed with TBRF do not have a recognized history of tick exposure. This is not surprising given the nocturnal nature of the *Ornithodoros* ticks, and the rapid transmission of TBRF *Borrelia* spp., as described above. All dogs had elevated temperatures, ranging from 103.2–104.6°F. Two cases presented with signs of neurologic disease including ataxia, tail tucking, and cranial nerve deficits. One dog also was reluctant to walk and had joint effusion, which resolved after treatment. TBRF *Borrelia* spp. have been shown to migrate to the nervous system in humans and mice, causing encephalitis, meningitis and neuritis, which likely explains the signs of neurologic disease in these dogs.[22] Together, these signs suggests a common, although nonspecific, clinical presentation of TBRF in dogs.

When the current and previously reported cases of TBRF in dogs are compared, there are several similarities and differences in hematology findings. All dogs were diagnosed during spirochetemic phases, therefore numerous spirochetes were observed on standard blood smear examination (Fig. 1). All dogs also had marked thrombocytopenia, when platelet counts were available.[15,16,21] Additional CBC findings varied between dogs but included mild stress leukograms, inflammatory changes, and mild non-regenerative anemia.[10] Several dogs displayed minimal CBC abnormalities other than marked thrombocytopenia and spirochetemia. While other hematologic abnormalities (i.e. inflammatory leukogram) might be expected given the bacteremia, they are not always observed, and thrombocytopenia appears to be the only consistent CBC finding in dogs with apparent spirochetemia. Cases of TBRF have only been characterized in animals with detectable spirochetemia and it is unknown if thrombocytopenia or clinical signs are present in animals during nonspirochetemic phases of TBRF.

Causes of thrombocytopenia in tick-borne diseases are often thought to be numerous and multifactorial. There have been several studies evaluating the interactions between TBRF *Borrelia* organisms and platelets in human medicine. In people infected with TBRF, it has been shown that *Borrelia hermsii* binds $\alpha_{IIb}\beta_3$ receptors on platelets, causing activation and accelerated removal of platelets.[23] In mice infected with TBRF spirochetes, spirochetes form complexes with platelets in the blood.[24] This allows indirect clearance of platelets while spirochetes are removed from circulation. In contrast with humans, there was no evidence of platelet activation in mice.[24] To the author's knowledge, similar studies have not yet been performed in dogs, but it possible that similar mechanisms could play a role in the development of thrombocytopenia.

Clinical signs and hematologic findings with TBRF can be similar to those of *Borrelia burgdorferi* sensu stricto, which can lead to clinical misdiagnosis of Lyme disease. This is particularly possible given that Lyme disease is considered a summer illness and the cases in this report presented between May and August. Two previous case reports and two cases presented here were positive by serologic testing for *B. burgdorferi* sensu stricto. TBRF *Borrelia* spp. have been shown to cross-react with Lyme disease *Borrelia* spp. with IFA.[12] However, cross-reactivity is not necessarily consistent between cases and testing modalities.

In humans, GlpQ and BipA antigens can be used as specific antigens for TBRF serology testing, as they are not present in Lyme *Borrelia* spp.[7,25] Serologic tests utilizing these antigens have been used to discriminate between the causative agents of Lyme disease and TBRF in humans.[26] To the author's knowledge, these tests have not yet been validated in veterinary animals; however, further research in this area would be warranted.

Three of the five cases presented here were seropositive to Rocky Mountain Spotted Fever (RMSF), caused by an intracellular gram negative coccobacillus (*Rickettsia rickettsii*). It is unclear if this represents a co-infection, previous exposure, exposure to nonpathogenic species, or an unidentified cross-reaction from the confirmed *Borrelia* infection.

To limit misdiagnosis and to aid in classification of *Borrelia*-induced diseases, advanced diagnostics might be indicated in animals suspected of having Lyme disease or TBRF. Confirmatory testing methods include western blot, culture, and PCR.[2] Culture from human patients requires large volumes of whole blood and is often unrewarding. Culture in animals has only been successfully completed via inoculation of mice to amplify the spirochetes, followed by DNA sequence analysis.[3] In human medicine, molecular diagnostics are not consistently performed when spirochetes are visualized on peripheral blood smears. It is the authors' recommendation that, as in humans, advanced diagnostic techniques are not necessary in dogs with visible spirochetemia. However, a lack of spirochetemia does not rule out TBRF. In areas endemic to TBRF, dogs with clinical signs of vector-borne disease would benefit from molecular diagnostics for TBRF, especially during nonspirochetemic phases. Commercially available vector-borne disease PCR tests do not typically target *Borrelia* species because Lyme *Borrelia* is not found in the blood at the time of clinical symptoms or signs. Therefore, the development of a novel, commercially available PCR technique that includes TBRF *Borrelia* detection will positively impact canine health.

Since TBRF is infrequently diagnosed in veterinary medicine, standardized treatment protocols are lacking. Published reports have been treated successfully with varying protocols of tetracycline administration.[10,15,16,21] In the current cases, all dogs were administered tetracyclines PO twice daily for 3–6 weeks. Case 4 was treated with minocycline[j] at 3.5 mg/kg (1.6 mg/lb) every 12 hours for 28 days. The remaining cases were treated with doxycycline[g] ranging from 4 to 7.5 mg/kg (1.8–3.4 mg/lb) PO, every 12 hours for 21–42 days (individual dosing is listed with each case). When follow-up data were available, CBC revealed no abnormalities or only mild (presumed rebound) thrombocytosis. With treatment, detectable spirochetemia and fever resolved in as early as 24 hours. Approximately 50% of human patients with TBRF develop a Jarisch-Herxheimer reaction, or worsening of clinical signs, with initial treatment.[27] This has not yet been observed in veterinary species, but dogs with high bacterial burdens should be monitored with initial treatment. The majority of dogs in this report recovered uneventfully; however, one dog was euthanized 6 months later for continued signs of neurologic disease. It is unclear if this was related to the spirochete infection.

There are several limitations of this case series. As a retrospective study, there is a lack of continuity between the clinical workup in each case. Additional tick-borne disease testing was not performed in all cases; therefore, concurrent vector-borne diseases cannot be entirely ruled out. When additional testing was pursued, coinfection or previous exposure to *Ehrlichia* and *Rickettsia* species were documented using serologic testing. Two dogs were also seropositive to *Borrelia burgdorferi*. However, cross-reactivity between TBRF and other *Borrelia* species has been described.[12] Further studies are indicated to characterize the cross-reactivity between serology tests for TBRF *Borrelia* organisms and *Borrelia burgdorferi*. The possibility of coinfection should always be considered in dogs with clinical signs of vector-borne disease. Molecular diagnostics were utilized in three of the cases included in this report to confirm non-Lyme *Borrelia* spp. (case 1, 2 and 3). Sequencing to confirm *Borrelia turicatae* was performed in two cases (case 1 and 2). A limitation of this study is that the spirochetes found in circulation in cases 4 and 5 were not confirmed using molecular diagnostics.

In this manuscript, we present additional cases of Tick-Borne Relapsing Fever with an overview of the disease. Dogs infected with TBRF *Borrelia* spp. share similar clinical signs and clinicopathologic data; however, some variation between infected dogs does occur. It is possible for animals with large numbers of circulating spirochetes to have minimal changes in CBC values. The limited abnormalities on CBC instrumentation reports might not prompt visual examination of a blood smear in a busy private practice setting. The presented cases emphasize the importance of performing blood smears in unhealthy dogs or those with any hematological abnormalities, including thrombocytopenia. Careful examination of a blood smear can allow for a rapid presumptive diagnosis of TBRF. Further research, development, and utilization of PCR for the detection of TBRF is warranted to aid in the detection of this disease during nonspirochetemic phases. With appropriate treatment, TBRF appears to be treatable. However, more studies are needed to determine the long-term outcomes for dogs.

Footnotes

[a] 4DX SNAP™ test· IDEXX Laboratories, Westbrook, ME
[b] Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX
[c] Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MT
[d] Roche Diagnostics, Indianapolis, IN
[e] CBS Scientific, Del Mar, CA
[f] Bio-Rad, Inc., Hercules, CA
[g] Eton Biosciences
[h] MacVector, Inc, Cary, NC
[i] oxycycline, Westward, Eatontown, NJ
[j] Veterinary Medical Research and Development. Pullman, WA
[k] Amoxicillin, Pfizer, New York City, NY
[l] Minocycline: Ranbaxy. Princeton, NJ
[m] Rimadyl: Pfizer. New York City, NY

Acknowledgments

References

1. Boyle WK, Wilder HK, Lawrence AM, et al. Transmission dynamics of *Borrelia turicatae* from the arthropod vector. PLoS Negl Trop Dis 2014;8:e2767.

2. Dworkin MS, Schwan TG, Anderson DE Jr, et al. Tick-borne relapsing fever. Infect Dis Clin North Am 2008;22:449–468, viii.

3. Schwan TG, Raffel SJ, Schrumpf ME, et al. Phylogenetic analysis of the spirochetes *Borrelia parkeri* and *Borrelia turicatae* and the potential for tick-borne relapsing fever in Florida. J Clin Microbiol 2005;43:3851–3859.

4. Fukunaga M, Ushijima Y, Aoki LY, et al. Detection of *Borrelia duttonii*, a tick-borne relapsing fever agent in central Tanzania, within ticks by flagellin gene-based nested polymerase chain reaction. Vector Borne Zoonotic Dis 2001;1:331–338.

5. Ras NM, Lascola B, Postic D, et al. Phylogenesis of relapsing fever *Borrelia* spp. Int J Syst Bacteriol 1996;46:859–865.

6. Halperin T, Orr N, Cohen R, et al. Detection of relapsing fever in human blood samples from Israel using PCR targeting the glycerophosphodiester phosphodiesterase (GlpQ) gene. Acta Trop 2006;98:189–195.

7. Schwan TG, Schrumpf ME, Hinnebusch BJ, et al. GlpQ: an antigen for serological discrimination between relapsing fever and Lyme borreliosis. J Clin Microbiol 1996;34:2483–2492.

8. Greene C. Infectious Diseases of the Dog and Cat, 4th ed. St. Louis, Missouri. Elsevier; 2011:431–447.

9. Schalm OW. Uncommon hematologic disorders - Spirochetosis, Trypanosomiasis, Leishmaniasis, Pelger-Huet anomaly. Canine Pract 1979;6:46–49.

10. Moreland KJ, Wilson EA, Simpson RB. Concurrent *Ehrlichia canis* and *Borrelia burgdorferi* infections in a Texas dog. J Am Anim Hosp Assoc 1990;26:635–639.

11. Coon D, Versalovic J. Tick-borne disease: a review of the more common entities found in the northeastern United States. Clin Microbiol Newsl 2002;24:9–14.

12. Dworkin MS, Schwan TG, Anderson DE Jr. Tick-borne relapsing fever in North America. Med Clin North Am 2002;86:417–433, viii–ix.

13. Schwan TG, Raffel SJ, Schrumpf ME, et al. Diversity and distribution of *Borrelia hermsii*. Emerg Infect Dis 2007;13:436–442.

14. Sato Y, Nakao M. Transmission of the Lyme disease spirochete, *Borrelia garinii*, between infected and uninfected immature *Ixodes persulcatus* during cofeeding on mice. J Parasitol 1997;83:547–550.

15. Whitney MS, Schwan TG, Sultemeier KB, et al. Spirochetemia caused by *Borrelia turicatae* infection in 3 dogs in Texas. Vet Clin Pathol 2007;36:212–216.

16. Breitschwerdt EB, Nicholson WL, Kiehl AR, et al. Natural infections with Borrelia spirochetes in two dogs from Florida. J Clin Microbiol 1994;32:352–357.

17. Raffel SJ, Battisti JM, Fischer RJ, et al. Inactivation of genes for antigenic variation in the relapsing fever spirochete *Borrelia hermsii* reduces infectivity in mice and transmission by ticks. PLoS Pathog 2014;10:e1004056.

18. Larsson C, Andersson M, Pelkonen J, et al. Persistent brain infection and disease reactivation in relapsing fever borreliosis. Microbes Infect 2006;8:2213–2219.

19. Walker RL, Read DH, Hayes DC, et al. Equine abortion associated with the *Borrelia parkeri-B. turicatae* tick-borne relapsing fever spirochete group. J Clin Microbiol 2002;40:1558–1562.

20. Evans NJ, Bown K, Timofte D, et al. Fatal borreliosis in bat caused by relapsing fever spirochete, United Kingdom. Emerg Infect Dis 2009;15:1331–1333.

21. Kelly AL, Raffel SJ, Fischer RJ, et al. First isolation of the relapsing fever spirochete, *Borrelia hermsii*, from a domestic dog. Ticks Tick Borne Dis 2014;5:95–99.

22. Cadavid D, Barbour AG. Neuroborreliosis during relapsing fever: review of the clinical manifestations, pathology, and treatment of infections in humans and experimental animals. Clin Infect Dis 1998;26:151–164.

23. Alugupalli KR, Michelson AD, Barnard MR, et al. Platelet activation by a relapsing fever spirochaete results in enhanced bacterium-platelet interaction via integrin alphaIIbbeta3 activation. Mol Microbiol 2001;39:330–340.

24. Alugupalli KR, Michelson AD, Joris I, et al. Spirochete-platelet attachment and thrombocytopenia in murine relapsing fever borreliosis. Blood 2003;102:2843–2850.

25. Lopez JE, Wilder HK, Boyle W, et al. Sequence analysis and serological responses against *Borrelia turicatae* BipA, a putative species-specific antigen. PLoS Negl Trop Dis 2013;7:e2454.

26. Wilder HK, Wozniak E, Huddleston E, et al. Case report: a retrospective serological analysis indicating human exposure to tick-borne relapsing fever spirochetes in Texas. PLoS Negl Trop Dis 2015;9:e0003617.

27. Dworkin MS, Anderson DE Jr, Schwan TG, et al. Tick-borne relapsing fever in the northwestern United States and southwestern Canada. Clin Infect Dis 1998;26:122–131.