Published in final edited form as:

Ticks Tick Borne Dis. 2014 June ; 5(4): 401–403. doi:10.1016/j.ttbdis.2014.01.007.

Detection of *Borrelia theileri* in *Rhipicephalus geigyi* from Mali

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Abstract

Disease burden within cattle is a concern around the world. Bovine borreliosis, one such disease, is caused by the spirochete *Borrelia theileri* transmitted by the bite of an infected *Rhipicephalus* (*Boophilus*) species tick. A number of species within the genus are capable of transmitting the agent and are found on multiple continents. Cattle in the West African nation of Mali are infested with 4 species of *Rhipicephalus* ticks of the subgenus *Boophilus*: *Rhipicephalus annulatus*, *Rhipicephalus microplus*, *Rhipicephalus decoloratus*, and *Rhipicephalus geigyi*. To date, no reports of *B. theileri* within Mali have been documented. We tested 184 *Rhipicephalus* spp. ticks by PCR that were removed from cattle at a market near Bamako, Mali. One tick, *R. geigyi*, was positive for *B. theileri*, which confirmed the presence of this spirochete in Mali.

Keywords

Bovine borreliosis; Tick; *Boophilus*; West Africa; Spirochete

Introduction

Borrelia theileri, the cause of bovine borreliosis, was identified in South Africa over 100 years ago and named in honor of Sir Arnold Theiler for his discovery of this spirochete (Laveran, 1903). Yet this species of spirochete is one of the least characterized pathogenic tick-borne borreliæ. Historically, the spirochete has been identified from Africa, Australia, and North and South America in the peripheral blood of large domestic mammals such as cows, goats, and sheep, which are parasitized by a few ixodid tick species known to carry and transmit this bacterium (Callow, 1967; Cutler et al., 2012; Smith et al., 1978, 1985; Theiler, 1904). The clinical signs of infection in cattle and other animals are mild and

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variable, but usually include fever and anemia (Smith et al., 1985). No isolates of *B. theileri* exist, and the few attempts to grow this spirochete in vitro have failed (Smith et al., 1985). Also, minimal DNA sequence data are available to differentiate this species from other species belonging to the genus. However, from the meager amount of DNA sequences available, *B. theileri* groups phylogenetically with the relapsing fever spirochetes, but unlike most other members of this group, this species is transmitted by hard (ixodid) ticks rather than soft (argasid) ticks (Theiler, 1905). This spirochete infects numerous tick tissues, as demonstrated by microscopic examination of Giemsa-stained organs of *R. microplus* (Smith et al., 1978). These one-host ticks also serve as the reservoir for *B. theileri*, given their efficient transovarial and transstadial transmission of the spirochete (Smith et al., 1978). Other members of this clade of relapsing fever-related spirochetes transmitted by hard ticks include *Borrelia miyamotoi* and *Borrelia lonestari* (Barbour et al., 1996; Fukunaga et al., 1995).

During our studies of tick-borne relapsing fever in Mali, we had the opportunity to collect ixodid ticks from cattle with the goal of testing them for infection with *B. theileri* (Schwan et al., 2012). Four *Rhipicephalus* (*Boophilus*) species of ticks infest cattle and other domestic stock in Mali: *Rhipicephalus annulatus* (Say), *Rhipicephalus decoloratus* (Koch), *Rhipicephalus microplus* (Canestrini), and *Rhipicephalus geigyi* (Aeschlimann & Morel) (Adakal et al., 2013; Smith et al., 1985; Theiler, 1905; Trees, 1978). Three of these ticks, *R. annulatus*, *R. decoloratus*, *R. microplus*, as well as *Rhipicephalus evertsi evertsi* (Neumann), are vectors of *B. theileri*, found in southern Africa and western Africa (Smith et al., 1985; Theiler, 1904; Trees, 1978). However, we are unaware of any reports of *B. theileri* within Mali or infecting the tick *R. geigyi*, whose role as a vector of the pathogens is uncertain (Walker et al., 2003). Therefore, our findings presented herein expand the knowledge of the distribution, tick association, and genetic characterization of *B. theileri* in Africa.

Materials and methods

Ticks were collected by hand from cattle on January 16 and June 19, 2010, at the Kati cattle market within the Koulikoro Region and June 20th, 2010, at the Korofina cattle market in Bamako in Mali, western Africa. Specimens were taken to the University of Bamako and tentatively identified to species based on published keys for ticks infesting domestic animals in Africa (Walker et al., 2003). The specimens were preserved in 70% ethanol and transported to the Rocky Mountain Laboratories, Hamilton, Montana, for further examination and testing. The ticks were removed from the ethanol and allowed to air dry. A whole tick, if less than 25 mg, or a 10–20-mg anterior section of larger ticks was placed into a microcentrifuge tube (Fisher Scientific Co, Pittsburgh, PA) and DNA extracted as previously described (Schwan et al., 2012). The mitochondrial 12S rRNA and 16S rRNA genes from selected ticks, and the *Borrelia* *flaB*, *glpQ*, *hpt*, and 16S rRNA genes were PCR-amplified and sequenced as previously reported using the primers listed in Table 1 (Schwan et al., 2012). Degenerate primers to the *hpt* gene were designed using Lasergene PrimerSelect (DNASTAR, Madison, WI). Sequences were aligned using ClustalW in the MEGA5 suite (<http://www.megasoftware.net>), and neighbor-joining trees with 1000 bootstrap replicates were produced.

Results

Through morphological and sequence comparisons, we identified 2 species among the 184 *Rhipicephalus* adults sampled, *R. geigy* and *R. decoloratus*. The infected tick and 20 additional specimens were selected for DNA sequence analysis, which yielded 19 *R. geigy* and 2 *R. decoloratus* based on 100% and 99% identities of their mt 12S rRNA gene (396 and 406 bp aligned, respectively). Two representative 12S rRNA sequences for *R. geigy* C7M and *R. decoloratus* C7F6, and the 16S rRNA sequence for *R. geigy* C7M are deposited in GenBank with accession numbers KF569939, KF569940, and KF569942, respectively.

PCR amplification with borrelia-specific primers resulted in products from a single *R. geigy* male (*R. geigy* C7M) from the Kati market, resulting in a 0.5% infection rate. The spirochete detected is herein referred to as *B. theileri* KAT. Sequences were generated for *B. theileri* KAT, which consisted of 1271 bp of 16S rRNA, 970 bp of *flaB*, 365 bp of *hpt*, and the entire 1002-bp *glpQ* gene. The borrelial 16S rRNA and *flaB* sequences were aligned with other borrelia sequences found in GenBank (Fig. 1A and 1B, respectively). These alignments included *B. theileri*, *B. lonestari*, and *B. miyamotoi*, as well as *Borrelia hermsii*, *Borrelia recurrentis*, found within a soft tick and louse, respectively, and the Lyme disease spirochete, *Borrelia burgdorferi*, as the outgroup. The 16S rRNA sequence alignment grouped *B. theileri* KAT and *Borrelia* sp. BR closely with 2 *B. theileri* sequences from GenBank, which identified our spirochete and *Borrelia* sp. BR as *B. theileri* (Fig. 1A) (Yparraguirre et al., 2007). The *B. theileri* KAT *flaB* sequence aligned closest with other *B. theileri flab* GenBank submissions, further confirming our identification (Fig. 1B). As *flaB* alignments with *B. theileri* KAT that included *B. theileri* from GenBank and *Borrelia* sp. BR resulted in minimal sequence overlap, a separate alignment compared relatedness of *B. theileri* KAT to *Borrelia* sp. BR. Similar to the 16S rRNA alignment, *Borrelia* sp. BR grouped closely with *B. theileri* KAT (data not shown). *Borrelia theileri* KAT *glpQ* and *hpt* genes were sequenced and submitted to GenBank (KF569938, KF569937, respectively), although no alignments were made due to a lack of comparable sequences from *B. theileri* elsewhere.

Discussion

This is the first observation of *B. theileri* within Mali and in *R. geigy*. *Rhipicephalus geigy* is genetically related to and overlaps in distribution with *R. decoloratus* and *R. annulatus*, known vectors of *B. theileri*. However, minimal data exist on the infection rates in *B. theileri* tick vectors (Estrada-Peña et al., 2006). The infection rate (0.5%) reported here is comparable to the study in Brazil which found less than 1% infected with *Borrelia* sp. BR in field-collected *R. microplus* (Yparraguirre et al., 2007). However, this sequence was not identified as *B. theileri* though it groups most closely with *B. theileri* KAT and other *B. theileri* in the phylogenetic analysis, leading us to conclude it is *B. theileri* (Fig. 1A and 1B).

Six additional *R. geigy* and one *R. decoloratus* ticks, all female, were also collected from the same bovine as the infected *R. geigy* C7M. Interestingly, none of those ticks had evidence of *B. theileri* infection by PCR amplification. *Rhipicephalus geigy*, like all the

species within the subgenus *Boophilus*, is a one-host tick, thus finding one infected tick upon a host suggests more may be present (Estrada-Peña et al., 2006). There are a few possible explanations for this result: (i) PCR of borrelial DNA from tick DNA extractions have lower sensitivity, likely due to inhibitory products or an overabundance of non-target DNA, leading to false negatives, (ii) *R. geigy* C7M may not yet have transmitted the organism to the cow, or (iii) the prevalence of *R. geigy* infected with *B. theileri* is below 1% and not enough ticks were sampled from the cow to find another positive.

PCR detection of the spirochete does not prove that *R. geigy* is a vector for *B. theileri*. *Borrelia* can be detected in ticks that are unable to transmit the organism; its presence may be transient and cleared without colonizing the tick. More research into the competence of *R. geigy* as a vector for *B. theileri* is needed. Tick-borne diseases of cattle are a great burden to farming communities around the world, particularly in rural Africa (Perry and Sones, 2007). Realizing the presence of *B. theileri* in Mali and the possible role of *R. geigy* as a vector may aid in the farmers efforts to bring healthier, more valuable products to market.

Acknowledgments

This work was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health. We thank Bob Fischer, Sandra Stewart, and Philip Stewart for reviewing the manuscript; Nafomon Sogoba and Sékou F. Traoré for field and administrative help; and Sory Coulibaly for allowing us to collect ticks at the cattle markets.

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Fig. 1.

Phylogram comparing the Malian *B. theileri* KAT to other borreliae based on 16S rRNA (A) and *flaB* (B). Sequence lengths were trimmed to 887 bp for 16S rRNA and 226 bp for *flaB* to include more sequences for alignment. Trees were inferred using the Neighbor-Joining method. The percentage of trees (>50%) in which the associated taxa clustered together in the bootstrap test of 1000 replicates are shown. Branch lengths are the number of base substitutions per site and were computed using the Maximum Composite Likelihood method. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA5. Taxon labels include the organism name and the GenBank accession number.

Table 1

Primers used for PCR amplification and sequencing.

Gene	Primer name	Sequence	Expected product size (bp)
16S rRNA	T50^a	GTTACGACTTCACCCCTCCT	1488
	FD3^a	AGAGTTTGATCCTGGCTTAG	
	Rec 4 ^a	ATGCTAGAACTGCATGA	
	Rec 9 ^a	TCGTCTGAGTCCCCATCT	
	16RnaL ^b	CTGGCAGTGCGTCTTAAGCA	
	16RnaR ^b	GTATTACCGTATCATTCTGATATAC	
	16S-2 ^a	TACAGGTGCTGCATGGTTGTCG	
	16S-1 ^a	TAGAAGTTCGCCTTCGCCTCTG	
<i>glpQ</i>	glpQF+1^a	GGGGTTCTGTACTGCTAGTGCCATTAC	1002
	Rev-2^a	CAATACTAAGACCAGTTGCTCCTCCGCC	
	glpQF-1 ^a	CAATTTTAGATATGTCTTTACCTTGTTGTTATGCC	
	Rev-1 ^a	GCACAGGTAGGAATGTTGGAATTTATCCTG	
<i>flaB</i>	Fla ans 5^a	TGTGATATCCTTTTAAAGAGACAAATGG	1284
	Fla alt 3^a	TCTAAGCAATGACAATACATATTGAGG	
	flaLL ^b	ACATATTCAGATGCAGACAGAGGT	
	flaRL ^b	GCAATCATAGCCATTGCAGATTGT	
	flaLS ^b	AACAGCTGAAGAGCTTGGAATG	
	flaRS ^b	CTTTGATCACTTATCATTCTAATAGC	
<i>hpt</i>	hptdegF^c	GCAGAYATTACAAGAGARATGG	433
	hptdegR^c	CYTCRTCACCCCATTTGAGTTCC	
Tick 16S rRNA	Tick16S+1^d	CTGCTCAATGATTTTTTAAATTGCTGTGG	480
	Tick16S-1^d	CCGGTCTGAACTCAGATCAAGT	
	Tick16S+2 ^d	TTGGGCAAGAAGACCCTATGAA	
	Tick16S-2 ^d	TTACGCTGTTATCCCTAGAG	
	Tick16S+3 ^d	ATACTCTAGGGATAACAGCGT	
	Tick16S-3 ^d	AAATTCATAGGGTCTTCTTGTC	
Tick 12S rRNA	SR-J-14199^e	TACTATGTTACGACTTAT	430
	SR-N-14594^e	AAACTAGGATTAGATACCC	

Amplification primers in bold; all primers used for sequencing.

^a Schwan et al. (2005).^b Barbour et al. (1996).

^c this study.

^d Black IV and Piesman (1994).

^e Kambhampati and Smith (1995).