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Detection of *Rickettsia massiliae* in *Rhipicephalus sanguineus* from the Eastern United States

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Abstract

We report the first evidence of *Rickettsia massiliae* in the brown dog tick, *Rhipicephalus sanguineus*, from the East Coast of the United States. As part of routine pathogen surveillance, DNA samples from ixodid ticks were tested for spotted fever group rickettsiae by nested PCR. A *R. massiliae*-positive tick was collected off a beagle mix recently rescued from North Carolina. Infection was confirmed by partial sequence analysis of the *htrA*, *gltA*, *ompB*, *ompA*, and *sca4* genes, which had 100% identity to a *R. massiliae* isolate from Arizona.

Key Words: *Rickettsia massiliae—Rhipicephalus sanguineus.*

Introduction

Rickettsia massiliae belongs to the spotted fever group rickettsiae. The bacterium was first isolated from a *Rhipicephalus* tick in France in 1990 (Beati et al. 1992). There have since been three confirmed human cases of spotted fever associated with *R. massiliae* infection, two in Europe and one in Argentina (Vitale et al. 2006; Parola et al. 2008; Garcia-Garcia 2010). However, because of its similar clinical manifestations to other rickettsial diseases and its cross-reactivity in serologic assays used for diagnosis, the actual prevalence and distribution of *R. massiliae* remains unknown (Beeler et al. 2011).

There have been two descriptions of *R. massiliae* detection in the United States. The bacterium has been isolated from a population of brown dog ticks, *Rhipicephalus sanguineus*, from eastern Arizona (Eremeeva et al. 2006), and has been detected in *R. sanguineus* from Los Angeles County, California, with corresponding serologic evidence of *R. massiliae* exposure in sick canines (Beeler et al. 2011). Here we report the first evidence that *R. massiliae* is present on the eastern seaboard of the United States.

Results

Rhipicephalus sanguineus ticks from Maryland and Virginia were processed alongside other ixodid ticks as part of routine pathogen prevalence surveys conducted from 2009–2011. Approximately 10,000 ticks were processed and screened for pathogens, including three *R. sanguineus* specimens. The ticks, which were collected off human and canine hosts, were identified morphologically and homogenized using a TissueLyser II bead mill (Qiagen, Valencia, CA), with 5-mm stainless steel beads. DNA was extracted from homogenized ticks using a MasterPure DNA Purification Kit (Epicentre Biotechnologies, Madison, WI), with a modified extraction procedure as described previously (Fornadel et al. 2011). DNA pellets were resuspended in 100 μL of molecular grade water and stored at -20°C.

DNA samples were tested for spotted fever group rickettsiae by nested polymerase chain reaction (PCR) using primers specific for a fragment of the rickettsial outer membrane protein B (*ompB*) gene (Choi et al. 2005). *Rickettsia conorii* was used as a positive control. Of the three adult *R. sanguineus* ticks screened, one male tested positive for spotted fever group rickettsiae. Interestingly, no product was attained when attempts were made to amplify a 602-bp fragment from the 5′ region of the rickettsial outer membrane protein A (*ompA*) gene for restriction digestion analysis using primers 190.70f and 190.701r (Roux et al. 1996). Consequently, the identity of the *Rickettsia* species was determined by sequencing the 267-bp *ompB* amplicon (GenBank accession no. JN408580). Additional partial sequences were obtained for the *ompA* gene, the 17-kDa antigen gene (*htrA*; JN408581), the citrate synthase (*gltA*) gene (JN408582), and the *Rickettsia* species "gene D" (*sca4*; JN408583; Tzianabos et al. 1989; Regnery et al. 1991; Sekeyova et al. 2001; Eremeeva et al. 2003). All PCR products used for sequencing were first purified using a QIAquick PCR purification kit (Qiagen).

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Table 1. Sequence Similarities Between the East Coast *Rickettsia massiliae* Specimen and *R. massiliae* Strains from European *R. sanguineus* Ticks

		Rickettsial genes							
		ompA[a]		*ompB*[b]		*gene D*[c]		*gltA*[d]	
Strain name	*Country of origin*	*GenBank no.*	*Similarities*	*GenBank no.*	*Similarities*	*GenBank no.*	*Similarities*	*GenBank no.*	*Similarities*
Bar29	Spain	U43792.1	130/130	AF123710.1	257/257	AF155056.1	592/592	U59720.1	350/350
GS	Greece	U43793.1	129/130	NA	NA	NA	NA	NA	NA
Mtu5	France	CP000683.1	129/130	CP000683.1	254/257	CP000683.1	587/592	CP000683.1	350/350

[a]Primers used in this study: 190.547f: CCT GCC GAT AAT TAT ACA GGT TTA and 190.701r: GTT CCG TTA ATG GCA GCA T (Ermeeva et al. 2003).
[b]Primers used in this study: 4362pOut: GTC AGC GTT ACT TCT TCG ATG C; 4836nOut: CCG TAC TCC ATC TTA GCA TCA G; 4496pIn: CCA ATG GCA GGA CTT AGC TAC T; and 4762nIn: AGG CTG GCT GAT ACA CGG AGT AA (Choi et al. 2005).
[c]Primers used in this study: D767f: CGA TGG TAG CAT TAA AAG CT and D1390r: CTT GCT TTT CAG TAT CAC (Sekeyova et al. 2001).
[d]Primers used in this study: RpCS.877P: GGG GGC CTG CTC ACG GCG G and RpCS.1258N: ATT GCA AAA AGT ACA GTG AAC A (Regnery et al. 1991).
Numbers represent the number of nucleotides in common.

The *R. massiliae*-positive tick was collected from a veterinarian's office in Virginia as part of the Fairfax County Health Department's tick surveillance program. The male tick was removed from a dog, a 32-lb beagle mix rescued from North Carolina, on January 23, 2011. Partial sequence comparisons of the *ompB* (257 bp), *ompA* (130 bp), *htrA* (201 bp), *gltA* (332 bp), and *sca4* (592 bp) rickettsial genes each revealed 100% sequence identity with the AZT80 *R. massiliae* strain isolated from Arizona (GenBank accession nos. DQ503428.1, DQ212707.1, DQ517444.1, DQ212705.1, and DQ503429.1, respectively). From our partial sequence analysis of five rickettsial genes from *R. massiliae* strains found in *R. sanguineus* ticks, both the East Coast specimen and the Arizona isolate share the most sequence identity with the Bar29 strain from Spain (Table 1).

Discussion

The identification of *R. massiliae* in a brown dog tick collected off a canine recently arrived in Virginia from North Carolina is the first report of this rickettsial species from the East Coast of the United States. This spotted fever group rickettsial organism appears to be the same genotype as an Arizona isolate also transmitted by *R. sanguineus* (Eremeeva et al. 2006). Partial sequence comparisons with five rickettsial genes revealed 100% identity between the Arizona isolate and our East Coast sample. Additionally, similarly to the East Coast sample, only two of five *R. sanguineus* ticks that tested positive for rickettsial DNA in the Arizona study produced an amplicon when tested with the *ompA* primer 190.70f (Eremeeva et al. 2006). *R. massiliae* from North America might have higher sequence variability in the 5′ region of the *ompA* gene than isolates from other continents, as Roux and colleagues (Roux et al. 1996) were able to amplify the *ompA* gene from the Mtu1, GS, and Bar29 strains of *R. massiliae* with primer 190.70f. Therefore, when screening ticks for *R. massiliae* in the United States, primers that target different regions of the *ompA* gene should be utilized.

The presence of *R. massiliae* on the eastern seaboard of the United States is of note because serological cross-reactivity exists among spotted fever group rickettsiae. Care should therefore be taken when diagnostic tests are performed, and *R. massiliae* should be considered as a potential source of spotted fever group rickettsiae infection when surveillance is conducted. Because *R. massiliae* has been associated with sick dogs in California (Beeler et al. 2011), and has been found infecting *R. sanguineus* tick populations living in close proximity to, and consequently with the potential to bite, humans in Arizona (Eremeeva et al. 2006), more work will need to be done to determine the prevalence and pathogenic potential of this rickettsia in the United States. Furthermore, the potential interactions of *R. massiliae* with other *Rickettsia* species, including *R. rhipicephali*, a common inhabitant of *R. sanguineus*, will need to be studied. Recognition of the potential of *R. massiliae* infection in *R. sanguineus* ticks from North Carolina will help further our understanding of emerging rickettsioses on the East Coast and throughout the country.

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Author Disclosure Statement

No competing financial interests exist.

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