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## Multistate Survey of American Dog Ticks (*Dermacentor variabilis*) for *Rickettsia* Species

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### Abstract

*Dermacentor variabilis*, a common human-biting tick found throughout the eastern half and along the west coast of the United States, is a vector of multiple bacterial pathogens. Historically, *D. variabilis* has been considered a primary vector of *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever. A total of 883 adult *D. variabilis*, collected between 2012 and 2017 from various locations in 12 states across the United States, were screened for rickettsial DNA. Tick extracts were evaluated using three real-time PCR assays; an *R. rickettsii*-specific assay, a *Rickettsia bellii*-specific assay, and a *Rickettsia* genus-specific assay. Sequencing of *ompA* gene amplicons generated using a seminested PCR assay was used to determine the rickettsial species present in positive samples not already identified by species-specific real-time assays. A total of 87 (9.9%) tick extracts contained *R. bellii* DNA and 203 (23%) contained DNA of other rickettsial species, including 47 (5.3%) with *Rickettsia montanensis*, 11 (1.2%) with *Rickettsia amblyommatis*, 2 (0.2%) with *Rickettsia rhipicephali*, and 3 (0.3%) with *Rickettsia parkeri*. Only 1 (0.1%) tick extract contained DNA of *R. rickettsii*. These data support multiple other

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#### Disclaimer

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#### Author Disclosure Statement

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contemporary studies that indicate infrequent detection of *R. rickettsii* in *D. variabilis* in North America.

## Keywords

*Dermacentor variabilis*; American dog tick; *Rickettsia rickettsii*; *Rickettsia bellii*; *Rickettsia parkeri*; *Rickettsia montanensis*

## Introduction

The American dog tick, *Dermacentor variabilis*, is a vector of various viral and bacterial pathogens, most notably *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever (RMSF). This tick is found throughout most of the eastern half and along the west coast of the United States, as well as parts of northern Mexico and southern Canada (Price 1954, Dergousoff et al. 2013, James et al. 2015). The number of reported cases of spotted fever rickettsiosis in the United States steadily increased from 1996 to 2016 (Biggs et al. 2016). At the same time, multiple field surveys of *D. variabilis* revealed a rarity or absence of *R. rickettsii* DNA detected among thousands of American dog ticks (Pretzman et al. 1990, Dergousoff et al. 2009, Stromdahl et al. 2011, Goddard et al. 2014, Gleim et al. 2016, Wood et al. 2016). In this context, infections caused by other less pathogenic *Rickettsia* species could be responsible for many cases of spotted fever rickettsiosis reported as “RMSF” (Openshaw et al. 2010). With *R. rickettsii* detected in less than 1% of the American dog ticks screened (Pretzman et al. 1990, Dergousoff et al. 2009, Stromdahl et al. 2011, Goddard et al. 2014, Wood et al. 2016, Trout Fryxell et al. 2017), it remains unclear why this pathogen is so elusive in *D. variabilis* populations. As suggested by investigations of *D. variabilis* and other *Dermacentor* species (Burgdorfer et al. 1981, Macaluso et al. 2002, Sakai et al. 2014), it is possible that the presence of other *Rickettsia* species affects the infrequency with which *R. rickettsii* is identified in American dog ticks. To further evaluate the occurrence of *R. rickettsii* and other *Rickettsia* species in this common human-biting tick, we screened collections of questing, adult *D. variabilis* from 12 U.S. states using real-time PCR.

## Materials and Methods

### Tick processing and DNA extraction

Between 2012 and 2017, a total of 883 adult *D. variabilis* ticks were collected from 39 counties and 1 independent city within 12 U.S. states: California, Georgia, Kansas, Kentucky, Maryland, Mississippi, Minnesota, New York, North Dakota, Pennsylvania, Virginia, and Washington. Ticks were identified morphologically using standard taxonomic keys and sent to the CDC preserved in ethanol (70–95%). DNA was extracted from ticks using a DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA) and eluted into a final volume of 200  $\mu$ L per the manufacturer’s instructions. For some specimens, the morphological identification was validated by sequencing of the tick 12S ribosomal RNA gene (Beati and Keirans 2001) or 16S ribosomal RNA gene (Black and Piesman 1994).

## Rickettsia screening

All extracts were screened for the presence of *R. rickettsii* and *Rickettsia bellii* DNA using two different real-time PCR assays, an *R. rickettsii*-specific TaqMan assay targeting the gene of hypothetical protein A1G\_04230, and an *R. bellii*-specific TaqMan assay targeting *gltA*, the citrate synthase gene (Kato et al. 2013, Hecht et al. 2016). The *R. rickettsii*-specific assay was performed as previously described except that 0.4  $\mu\text{M}$  of each primer and 12.5  $\mu\text{L}$  QuantiTect Multiplex PCR Master Mix (QIAGEN) were used in each reaction. Four microliters of template DNA was used in each *R. rickettsii*-specific reaction and 5  $\mu\text{L}$  of template DNA was used in the *R. bellii*-specific reactions. All real-time PCRs were performed in duplicate on a BioRad CFX 96 thermal cycler with a final reaction volume of 25  $\mu\text{L}$ . We considered samples positive if one of the duplicates had a cycle threshold (Ct) <40. Two sets of negative controls and one set of positive controls were included on each plate, where water was used as the negative nontemplate control and DNA from cultured *R. rickettsii* or an *R. bellii* plasmid were used as positive controls (Hecht et al. 2016).

Additional screening was performed on all 883 tick extracts to identify other rickettsial species present using a *Rickettsia* genus-specific TaqMan real-time assay targeting the *gltA* gene (Denison et al. 2014). The reactions were performed as described using 0.2  $\mu\text{M}$  of each primer and 4  $\mu\text{L}$  of DNA. Samples positive by the *gltA* real-time assay were further screened using a seminested PCR targeting the *ompA* gene of spotted fever group *Rickettsia* (Regnery et al. 1991, Ereemeeva et al. 1994, Roux et al. 1996). PCRs were performed using 1  $\mu\text{M}$  of each primer (Rr190.70, Rr190.602, Rr190.701), 10  $\mu\text{L}$  of Taq PCR Master Mix (QIAGEN), 2  $\mu\text{L}$  of sample DNA in the primary reaction or 4  $\mu\text{L}$  of the primary reaction product in the secondary reaction, and water to bring the final reaction volume to 20  $\mu\text{L}$ . DNA amplicons were visualized on 1.5% agarose gels containing 0.1  $\mu\text{g}/\text{mL}$  ethidium bromide. Amplicons were extracted and purified using the Promega Wizard SV Gel and PCR Clean-up System (Promega, Madison, WI). Products were bidirectionally sequenced using a BigDye Terminator v3.1 kit on an ABI 3130xl genetic analyzer (Applied BioSystems, Carlsbad, CA) and assembled using Geneious version 7.0.4. (<http://geneious.com>, Kearse et al. 2012). A BLAST analysis comparing the assembled sequences with sequences available in GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) identified the *Rickettsia* species present in the tick sample.

## Results

Rickettsial DNA was detected using the *Rickettsia* genus-specific *gltA* real-time assay in 203 (23%) of the 883 total DNA extracts. *Rickettsia*-positive tick extracts were identified from each state included in this survey (Table 1). Of these, 75 (36.9%) produced *ompA* amplicons that could be used for sequencing. Attempts to amplify additional gene targets were unsuccessful, with the rickettsial DNA concentration being too low in the other samples for sequencing. Amplicons from 63 of these 75 samples were successfully sequenced, identifying 11 (1.2%) samples positive for DNA of *Rickettsia amblyommatis*, 47 (5.3%) for *Rickettsia montanensis*, 2 (0.2%) for *Rickettsia rhipicephali*, and 3 (0.3%) for *Rickettsia parkeri* (Table 1). There were 87 (9.9%) tick samples positive for *R. bellii*. Only one (0.1%) tick, a male specimen collected from Muhlenberg County in Kentucky, contained

DNA of *R. rickettsii*. This same tick was the only coinfecting tick sample identified, and was found to contain both *R. bellii* and *R. rickettsii* DNA.

## Discussion

Specimens evaluated in this study were procured from general acarological surveys rather than collection efforts that specifically targeted *D. variabilis*. For this reason, sample sizes varied greatly and were often represented by fewer than 50 ticks from a particular county, precluding generalizations for individual regions or states. Nonetheless, we detected DNA of *R. rickettsii* in only 1 (0.1%) of the 883 *D. variabilis* ticks sampled from a total of 40 jurisdictions in 12 U.S. states. By comparison, we identified rickettsial agents of lesser or unknown pathogenicity, including *R. bellii*, *R. montanensis*, *R. parkeri*, and *R. rhipicephali* in 150 (17%) of the specimens.

The infrequent detection of *R. rickettsii* among questing adult American dog ticks is consistent with many other contemporary studies that either failed to identify this pathogen in *D. variabilis* or found very low rates of infection (Wikswa et al. 2008, Dergousoff et al. 2009, Moncayo et al. 2010, Williamson et al. 2010, Fritzen et al. 2011, Stromdahl et al. 2011, Goddard et al. 2014, Henning et al. 2014, Nadolny et al. 2014, Pagac et al. 2014, Gleim et al. 2016, Mitchell et al. 2016, Wood et al. 2016). Despite the low infection frequency of *R. rickettsii* in *D. variabilis* ticks collected in nature, this tick has been shown to be an efficient vector for *R. rickettsii* (Maver 1911, Burgdorfer 1975) and several isolates of *R. rickettsii* have been made from wild *D. variabilis*, proving that this tick does indeed become infected with *R. rickettsii* in the environment (Cox 1941, Karpathy et al. 2007). In addition, many of the human RMSF cases reported each year in the United States are from areas where *D. variabilis* is endemic (Biggs et al. 2016). Previously, it was reported that *R. rickettsii* has a lethal effect on immature *Dermacentor andersoni* ticks that significantly diminished the number of infected adult ticks (Niebylski et al. 1999), and it was proposed that this deleterious effect may explain the low prevalence of infected ticks in nature. However, Schumacher et al. (2016) recently demonstrated that in the laboratory *R. rickettsii* had no detrimental effects on the survival of infected *D. variabilis* ticks at any life stage.

Approximately 10% of the ticks evaluated in this study contained DNA of *R. bellii*, although the frequency of infection varied considerably state to state. For example, 88% of the 69 ticks collected from one site in Yolo County in northern California contained *R. bellii*, whereas none of the 196 ticks collected from Kansas, Maryland, Minnesota, New York, and North Dakota were infected with this species. *R. bellii* is often missed in prevalence studies because many of the molecular assays used to screen for *Rickettsia* target the rickettsial *ompA* gene, which is absent in *R. bellii* (Ogata et al. 2006). Inclusion of species-specific screening for *R. bellii* provides a more complete picture of the rickettsial species present within these tick populations and enabled us to identify the dually infected tick from Kentucky (Table 1). This could be important when considering the infrequency of *R. rickettsii* in some populations of *D. variabilis*. One mechanism by which *R. bellii* is maintained in tick populations is by transovarial transmission from an infected female to her offspring, and a primary *R. bellii* infection has been shown to inhibit the transovarial transmission of a secondarily acquired *Rickettsia* species (Sakai et al. 2014). Therefore, the

high frequency of *R. bellii* in California may play a significant inhibitory role in the maintenance of other pathogenic *Rickettsia* species and reduce significantly the occurrence of *R. rickettsii* in *D. variabilis* populations in that area. Although *R. bellii* may play an important inhibitory role within this tick population in Yolo County, California, this is a regionally specific finding that does not apply to the other tick populations where no *R. bellii* was identified.

*R. montanensis* has also been found to inhibit transovarial transmission of a secondarily acquired *Rickettsia* species in *D. variabilis* ticks (Macaluso et al. 2002). Similar to *R. bellii*, the frequency of *R. montanensis* varied greatly among tick samples, from ~32% of the 72 ticks collected from 2 counties in Minnesota to only 2.9% among the remaining 811 specimens collected from 11 other states. Infection rates of between 0% and 10.5% have been described in previously published studies (Wikswa et al. 2008, Dergousoff et al. 2009, Moncayo et al. 2010, Williamson et al. 2010, Fritzen et al. 2011, Stromdahl et al. 2011, Henning et al. 2014, Nadolny et al. 2014, Pagac et al. 2014, Gleim et al. 2016, Mitchell et al. 2016, Wood et al. 2016). *R. montanensis*, a potential human pathogen (McQuiston et al. 2012), may play an inhibitory role in the maintenance of other pathogenic *Rickettsia* species in these Minnesota tick populations.

*R. amblyommatis* was identified in ~1–8% of the ticks collected from Kentucky, New York, and Virginia, and absent from 533 specimens collected from the remaining nine states, notably the northern and western states of California, Minnesota, North Dakota, and Washington where the ranges of *Amblyomma americanum*, the primary host of *R. amblyommatis* in the United States does not extend. Although *D. variabilis* is not the primary host for *R. amblyommatis*, it has been previously identified in 0.4–2.5% of ticks collected from Georgia, Kentucky, Tennessee, Texas, and Virginia (Moncayo et al. 2010, Williamson et al. 2010, Fritzen et al. 2011, Stromdahl et al. 2011, Henning et al. 2014, Gleim et al. 2016), and one recent study suggests that *R. amblyommatis* may cause mild or subclinical infections in humans (Apperson et al. 2008).

*R. rhipicephali* was identified in only two specimen: one from North Dakota and one from Washington. To our knowledge this rickettsial species has not been previously reported in *D. variabilis* from these states. *R. parkeri* was identified in ~2.4% of the ticks collected from Muhlenberg County, Kentucky. *R. parkeri* is the causative agent of a spotted fever rickettsiosis found throughout the southern United States and transmitted predominantly by the Gulf Coast tick, *Amblyomma maculatum* (Paddock and Goddard 2015). Previous surveys have identified *R. parkeri* in *D. variabilis* ticks from Kentucky (0.6%), Virginia (0.7%), and Texas (2.3%) (Williamson et al. 2010, Fritzen et al. 2011, Henning et al. 2014), and recently *D. variabilis* has been shown to both acquire and transstadially transmit *R. parkeri* in a laboratory setting (Harris et al. 2017). At this time, it remains unclear what role this tick species plays in transmitting *R. parkeri* in nature; nonetheless, in the regions of the United States where *R. parkeri* has been identified, the frequencies with which this pathogen occurs in *D. variabilis* exceeds those observed for *R. rickettsii* (Williamson et al. 2010, Fritzen et al. 2011, Henning et al. 2014).

The well-documented scarcity of *R. rickettsii* in *D. variabilis* populations calls into question just how significant a role this tick plays in the maintenance of *R. rickettsii*; it thus becomes important to consider the role of other tick species in the disease ecology of RMSF. The most recent outbreak of RMSF in the United States occurred in eastern Arizona where the tick species *Rhipicephalus sanguineus* sensu lato was identified as the vector of *R. rickettsii* (Demma et al. 2005). This tick species found throughout the United States is typically not an aggressive human-biting tick; however, incidents of *Rh. sanguineus* biting humans have been reported in Alabama, Georgia, Kansas, Kentucky, Louisiana, Maryland, New Jersey, North Carolina, Oklahoma, Pennsylvania, Rhode Island, South Carolina, Texas, and Virginia (Estrada-Pena and Jongejan 1999, Stromdahl et al. 2011). Of note, North Carolina is the state with the highest number of reported spotted fever group rickettsioses cases from 2000 to 2012 (Openshaw et al. 2010, Drexler et al. 2016).

Other tick species for consideration as a bridging vector include *Amblyomma americanum* and *Haemaphysalis leporispalustris*. Parker reported isolating *R. rickettsii* from *H. leporispalustris*, collected from the Bitter Root Valley of Montana in 1951 (Parker et al. 1951), and it was later isolated from *H. leporispalustris* collected from Costa Rica (Fuentes et al. 1985). Although this tick has not been associated with any human cases in the United States and is generally not considered a frequent human biter (Merten and Durden 2000), *H. leporispalustris* has been identified as a competent vector of *R. rickettsii* under laboratory conditions (Freitas et al. 2009) and could play a role in enzootic maintenance of *R. rickettsii*. Lastly, *A. americanum* is not only a competent vector of *R. rickettsii* under laboratory conditions (Levin et al. 2017), it has also been implicated as the vector in a human case of RMSF in North Carolina (Breitschwerdt et al. 2011). Although reports of *R. rickettsii* in U.S. populations of *A. americanum* are scarce (Berrada et al. 2011), this aggressive human-biting tick species is found throughout the eastern half of the United States and regularly accounts for a majority of the ticks removed from humans in surveys of southeastern states (Estrada-Pena and Jongejan 1999, Gleim et al. 2016, Mitchell et al. 2016). The role of these other tick species in the maintenance of *R. rickettsii* may be greater than previously thought and our efforts to better understand the disease ecology of RMSF should include further investigation into alternative tick vectors.

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**Table 1.**

**RICKETTSIA SCREENING RESULTS DIVIDED BY STATE**

State of collections (no. of counties/ unincorporated cities surveyed) <sup>a</sup>	Year(s) of collection	Total no. of ticks positive for rickettsial DNA/ total no. of ticks tested (%)	Total No. of ticks positive (%)						
			Rickettsia amblyommatis	Rickettsia montanensis	Rickettsia rhipicephali	Rickettsia parkeri	Rickettsia bellii	Rickettsia rickettsii	
California (1)	2013, 2015	32/69 (46%)	0	0	0	0	0	61 (88.4%)	0
Georgia (5)	2015, 2017	7/71 (9.8%)	0	3 (4.2%)	0	0	0	2 (2.8%)	0
Kansas (1)	2012	48/86 (56%)	0	9 (10.5%)	0	0	0	0	0
Kentucky (2)	2014–2016	23/124 (19%)	1 (0.8%)	3 (2.4%)	0	0	3 (2.4%)	11 (8.9%)	1 (0.8%)
Maryland (1)	2014	1/3 (33%)	0	0	0	0	0	0	0
Minnesota (2)	2015	26/72 (36%)	0	23 (31.9%)	0	0	0	0	0
Mississippi (2)	2013–2015	2/51 (3.9%)	0	0	0	0	0	1 (2.0%)	0
New York (1)	2014–2015	2/12 (17%)	1 (8.3%)	1 (8.3%)	0	0	0	0	0
North Dakota (11)	2016	2/23 (8.7%)	0	1 (4.3%)	1 (4.3%)	0	0	0	0
Pennsylvania (1)	2014–2015	21/55 (38%)	0	2 (3.6%)	0	0	0	1 (1.8%)	0
Virginia (2)	2014–2015	25/214 (12%)	9 (4.2%)	3 (1.4%)	0	0	0	4 (1.9%)	0
Washington (11)	2012, 2014–2015	14/103 (14%)	0	2 (1.9%)	1 (1.0%)	0	0	7 (6.8%)	0
Total		203/883 (23%)	11 (1.2%)	47 (5.3%)	2 (0.2%)	3 (0.3%)	87 (9.9%)	1 (0.1%)	

<sup>a</sup> A list of the counties: California (Yolo), Georgia (Cobb, Coweta, Douglas, Gwinnett, Rockdale), Kansas (Osage), Kentucky (Edmonson, Muhlenberg), Maryland (Frederick), Mississippi (Oktibbeha), Minnesota (Beltrami, Hubbard), New York (Suffolk), North Dakota (Bottineau, Burleigh, Grant, Kidder, McHenry, Mercer, Mountrail, Nelson, Pierce, Walsh, Ward), Pennsylvania (Adams), Virginia (City of Chesapeake, Prince William), and Washington (Benton, Franklin, Grant, Kittitas, Klickitat, Lincoln, Okanogan, Spokane, Yakima, Walla Walla, Whitman).