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Spotted Fever Group Rickettsiae in Multiple Hard Tick Species from Fairfax County, Virginia

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Abstract

Spotted fever group rickettsiosis (SFGR) is a potentially fatal disease that has displayed increasing incidence in the United States in recent years. The most well-known and severe type of this disease is Rocky Mountain spotted fever, but there are other mild forms that occur. Recently, human infection with *Rickettsia parkeri* has been reported and linked with the tick *Amblyomma maculatum*. In 2010, a population of *R. parkeri*-infected *A. maculatum* was discovered in Fairfax County, Virginia, leading to increased surveillance of tick species. In this study, we report the presence of *R. parkeri* in *Rhipicephalus sanguineus*, *Haemaphysalis leporispalustris*, and *Dermacentor variabilis* in Fairfax County. *R. parkeri* was discovered in two *Rh. sanguineus*, one *H. leporispalustris*, and 17 *D. variabilis*. These findings suggest that spillover infections of *R. parkeri* may be occurring in tick species not typically associated with this pathogen; however, vector competence studies need to be conducted to determine if these tick species can serve as potential vectors for human SFGR.

Key Words: *Rickettsia parkeri—Rhipicephalus sanguineus—Haemaphysalis leporispalustris—Dermacentor variabilis.*

Introduction

Spotted fever group (SFG) rickettsiae are a class of bacterial pathogens vectored by arthropods (typically ticks) capable of causing severe disease (Parola et al. 2005). The most well-known member of this class of rickettsiae is *Rickettsia rickettsii,* the causative agent of Rocky Mountain spotted fever (RMSF), which was first discovered at the turn of the 20th century (Ricketts 1906). In the subsequent century since the discovery of *R. rickettsii*, numerous other SFG rickettsiae have been discovered, illustrating the diversity among this group of bacterial pathogens. Despite the detailed histories of numerous members of the SFG rickettsiae, it was not until recently that many of these organisms were considered pathogenic and conclusively linked to human infection (Raoult 2004).

One of the most important SFG rickettsiae recently recognized as pathogenic is *Rickettsia parkeri*, which was a well-known zoonotic infection dating as far back as the 1930s (Parker et al. 1939), but was first recognized as a human pathogen in 2002, when an individual developed mild spotted fever rickettsiosis (SFR) (Paddock et al. 2004). Since its initial discovery as a causative agent for SFR, *R. parkeri* has been implicated in 12 cases, with a strong likelihood of contributing to many more cases of the disease (Paddock et al. 2008).

In 2010, a population of *R. parkeri*–infected *Amblyomma maculatum* was discovered at a closed landfill in Fairfax County, Virginia (Fornadel et al. 2011). The discovery of *R. parkeri* and *A. maculatum* in this landfill led to an expansion of tick surveillance efforts in Fairfax County, with particular attention paid to surveillance for rickettsial pathogens. Fairfax County is at a geographical intersection of the ranges of many tick species, with established populations of *Ixodes scapularis, Dermacentor variabilis,* and *Amblyomma americanum* (Fornadel et al. 2011). These species are often collected sympatrically at a number of locations, enabling the possibility of co-feeding and subsequent spillover of pathogens. Pathogen spillover, although commonly considered as a transfer of pathogen between reservoir hosts and other species, could also occur in vectors, which may lead to subsequent transmission to additional reservoirs or incidental hosts, thus propagating pathogen transmission (Daszak et al. 2000).

During the past decade, reported incidence rates of RMSF and rickettsial diseases have increased dramatically, underscoring the

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need for further investigation of SFG rickettsiae in tick populations (Openshaw et al. 2010). In this study, we report the current state of rickettsial pathogens in local tick populations, with particular attention paid to the discovery of *R. parkeri* in *Rhipicephalus sanguineus* and *Haemaphysalis leporispalustris*. Additionally, the presence of SFG rickettsiae in *D. variabilis* in Fairfax County is detailed.

Materials and Methods

Tick collections

Surveillance was performed from January, 2012, through December, 2012. Ticks were collected using either drag cloths (March–November) at the landfill location or carbon dioxide traps (January–December) at specific locations throughout Fairfax County. *D. variabilis* and *H. leporispalustris* were collected along with *A. maculatum, A. americanum*, and *I. scapularis*. *Rh. sanguineus* were provided to the health department via a veterinary clinic in November and December as well as collections from an infestation at a residence in December.

Sample processing

A modified version of the MasterPure Complete DNA Purification Kit (Epicentre Biotechnologies, Madison, WI) was used to extract DNA for molecular processing. Individual ticks were placed into a 2-mL round-bottomed tube with 50 μL of Tissue and Cell Lysis Solution and a 5-mm stainless steel bead. Samples were placed in the TissueLyser II (Qiagen, Valencia, CA) for 3 min at 30 Hz. The samples were then centrifuged at 13,200 rpm for 1 min. Following centrifugation, 250 μL of Tissue and Cell Lysis solution and 2.5 μL of Proteinase K (20 μg/μL) were added to the sample tubes, beads were removed, and samples were incubated in a 65°C water bath for 60 min. Samples were placed on ice for 5 min, and 150 μL of MPC Protein Precipitation Reagent was added. The samples were vortexed for 10 s, and debris was pelleted by centrifugation for 10 min at 14,000 rpm at 4°C. The supernatant was removed and transferred to a new 1.5-mL microcentrifuge tube, 500 μL of ice-cold isopropanol was added, and the DNA pelleted by centrifugation at 14,000 rpm at 4°C. The supernatant was discarded following centrifugation, and samples were washed two times with 500 μL of ice-cold 75% ethanol. The samples were allowed to dry overnight and were resuspended in molecular-grade water.

Molecular diagnostics of SFG Rickettsia

Tick DNA extractions were screened for SFG *Rickettsia* using a nested-PCR assay that amplified the rickettsial outer membrane protein A (*ompA*) gene (Blair et al. 2004). PstI endonuclease restriction fragment length polymporphism (RFLP) analysis was used on *ompA*-positive samples to determine rickettsial species (Roux et al. 1996). A 25-μL reaction mixture containing 20 μL of *ompA* product, 1 μL of PstI (Invitrogen, Grand Island, NY), 2.5 μL 10× NEBuffer 3, and 0.3 μL of bovine serum albumin (New England BioLabs, Ipswitch, MA) was incubated at 37°C to complete the reaction. All positive samples were sequenced using *ompA*-specific primers 190.FN1 and 190.RN1 to confirm results and assess any variability between samples (Blair et al. 2004).

A rickettsial outer membrane protein B (*ompB*) gene PCR diagnostic was performed on the *R. parkeri ompA*-positive *Rh. sanguineus* and *H. leporispalustris* as a confirmatory step since these results were unexpected. All positive samples were sequenced using *ompB*-specific primers Rc.rompB.4,496p and Rc.rompB.4,762n to confirm results and assess any variability between samples (Choi et al. 2005).

Results

A total of 2396 samples from 2012 collections were screened for SFG *Rickettsia* (Table 1). A total of 17 *D. variabilis* were positive for *R. parkeri*, 0.96% of all *D. variabilis* screened for SFG *Rickettsia*. A total of two *Rh. sanguineus* (3.33%), 12 *A. maculatum* (25.53%), and one *H. leporispalustris* (20%) were also positive for *R. parkeri*. Additionally, four *D. variabilis* were positive for *R. montanensis* (0.18%), and one *D. variabilis* was positive for *R. amblyommii.*

Sequence results for a 267 base pair *ompB* gene for the *Rh. sanguineus* and *H. leporispalustris* samples were confirmed to be *R. parkeri*, with 99% identity to known *R. parkeri* sequences (GenBank accession no. FJ644549). Identity between *ompB* genes of *Rh. sanguineus*, *H. leporispalustris*, and selected *D. variabilis* was between 97% and 100%.

Discussion

The identification of *R. parkeri* in an unfed *H. leporispalustris* marks the first time that this rickettsial species has been identified in this tick species. Additionally, *R. parkeri* was discovered in *Rh. sanguineus*, which has been previously reported in one tick in Texas (Williamson et al. 2010). To our

Table 1. Infection Rates of *Rickettsia* in Various Tick Species in Fairfax County, Virginia

Species	*Male*	*Female*	*Nymph*	*Larva*	*Overall infection rate*
	Total number tested (number positive for *R. parkeri*)				
Dermacentor variabilis	1182 (9)	1092 (8)	0 (0)	0 (0)	0.75%
Rhipicephalus sanguineus	34 (2)	26 (0)	0 (0)	0 (0)	3.33%
Haemaphysalis leporispalustris	0 (0)	0 (0)	0 (0)	5 (1)	20%
Amblyomma maculatum	18 (6)	31 (6)	2 (0)	0 (0)	23.53%
	Total number tested (number positive for *R. montanensis*)				
Dermacentor variabilis	1182 (3)	1092 (1)	0 (0)	0 (0)	0.18%
	Total number tested (number positive for *R. amblyommii*)				
Dermacentor variabilis	1182 (1)	1092 (0)	0 (0)	0 (0)	0.04%

knowledge, this marks the second time this pathogen has been reported in *Rh. sanguineus.* The presence of *R. parkeri* in these various tick species is of particular note because of the increasing trend of mild SFR cases in the United States (''Rocky Mountain Spotted Fever—Statistics and Epidemiology'' 2012). Determining the expanding maintenance and transmission cycles of *R. parkeri* could provide avenues for further study on the increasing trend of this disease.

The presence of *R. parkeri* in *D. variabilis* has been previously reported, so its discovery in these samples appears to confirm its ability to infect this particular tick species (Williamson et al. 2010, Fornadel et al. 2011). Previously, it was suggested that the presence of *R. parkeri* in *D. variabilis* in Fairfax County might be a result of spillover due to cofeeding with infected *A. maculatum* because all *R. parkeri*–infected samples were collected at the same landfill location, and this suggestion appears to have merit because the majority of *R. parkeri*–infected *D. variabilis* were co-collected with *R. parkeri*–infected *A. maculatum*. Of the 17 *R. parkeri*–infected *D. variabilis*, 14 were collected at the landfill location, one from the Lorton site, and two from a veterinary clinic. The landfill and Lorton collection sites are the only sites regularly surveyed in the county that contain *R. parkeri*–infected *A. maculatum*, so it is interesting to note that the only infected *D. variabilis* were collected from these sites. Although the idea that incidental infection from host sharing is plausible, it should be noted that there is no information on the maintenance of this particular rickettsial pathogen in *D. variabilis* or if there has always been some low-level infection rate that has been previously unrecognized.

None of the *D. variabilis* or other ticks screened was infected with *R. rickettsii*, a result that is consistent with screenings conducted in years past (Stromdahl et al. 2011, D.E. Norris, unpublished data). Although there were no *R. rickettsii*–infected *D. variabilis,* there were four that were positive for *R. montanensis*, a known pathogen associated with *D. variabilis* in the mid-Atlantic region and a recently described causative agent of SFR in humans (Ammerman et al. 2004, McQuiston et al. 2012). Additionally, there was one *R. amblyommii*–positive sample. Although an interesting finding because this pathogen is not normally associated with *D. variabilis*, this observation has also been reported in North Carolina (Smith et al. 2010). This particular SFG *Rickettsia* has also been hypothesized to be a cause of human rickettsiosis (Apperson et al. 2008).

Rh. sanguineus is traditionally considered a parasite of dogs and is generally associated with infestations in homes and kennels (Fox and Sykes 1985, Gray et al. 2013). With the exception of eight ticks, the samples that were collected in this study were mainly provided from a veterinary clinic in the county. These samples were removed from dogs and saved for future identification and analysis by the local health department and our laboratory. Both of the samples that tested positive for *R. parkeri* were provided by the veterinary clinic. It is unknown how the animals that presented with infestation contracted these ticks, because they are not thought be free-living in the county. It is likely that these ticks might have taken a previous blood meal on a *R. parkeri*–infected animal to acquire the initial infection and then were subsequently collected after attaching to a dog in the county, although any suggestion about the ecology of infection is pure speculation.

H. leporispalustris is a known vector for *R. rickettsii* that is considered a parasite of lagomorphs (Bishopp and Trembley 1945). Over the course of 2012, only five of these ticks were collected, which is likely the result of the typically nidicolous behavior of these ectoparasites. The ticks were collected by CO_2 traps in the same area as *R. parkeri*–infected *A. maculatum* and *D. variabilis,* which might provide insight into how they became infected. *A. maculatum* has been shown in the laboratory to successfully molt when fed on lagomorphs, but there is little evidence to suggest that they naturally feed on these species (Koch and Hair 1975, Estrada-Pena et al. 2005). Larvae from both species utilize birds as hosts, but they appear to be refractory for *R. parkeri*, making it unlikely that infection could occur through host sharing (Keirans and Durden 1998, Durden et al. 2001, Eisen et al. 2004, Moraru et al. 2013). Host sharing could occur with *D. variabilis*, because it is known to parasitize rabbits, making a potential bridge for *R. parkeri* to *H. leporispalustris* (Saliba et al. 1966).

The discovery of *R. parkeri* in tick species not normally associated with the pathogen necessitates further investigation. Although there appears to be some geographic coincidence that supports the hypothesis of spillover due to host sharing, additional study into the ecology of these infections would provide more definitive evidence as to the origin of these infections and if they facilitate a complete life cycle for the pathogen. It remains unclear whether these tick species are contributing to the increasing rates of mild SFR disease, be that as bridge vectors for traditionally recognized hosts or direct infection of humans, and vector competence studies are necessary to determine their involvement.

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Author Disclosure Statement

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