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Short communication

Spotted fever group Rickettsiae in Ticks from Missouri

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ABSTRACT

Tick-borne rickettsioses pose a major health threat among vector-borne infections in Missouri but there are some uncertainties regarding the vector competence and range of tick species, as well as the virulence of certain bacterial species. A survey was developed and implemented to assess local healthcare practitioners' awareness of the prevalence and diagnosis of tick-borne diseases. In addition, ticks collected from rural areas of St. Louis County, Missouri, were evaluated to detect spotted fever group (SFG) rickettsiae and to determine the most common tick species present. Physician responses showed a good general awareness of tick-borne diseases and antibiotic choices but responses varied regarding length of treatment, the most common tick-borne diseases, and tick vectors. No new tick vectors were collected in the area; *Rickettsia amblyommatis* was the predominant SFG species, and it was detected in *Amblyomma americanum*, *Dermacentor variabilis*, and*Ixodes scapularis*; *Rickettsia montanensis* was detected in *D. variabilis*. The high prevalence of *Rickettsia amblyommatis* in these ticks suggests that there is a high risk of exposure to this SFG rickettsial species to humans and that it may be providing some cross-protective immunity to *R. rickettsii*.

1. Introduction

Rickettsia rickettsii is the most virulent of all known spotted fever group (SFG) rickettsiae and is the causative agent of Rocky Mountain spotted fever (RMSF) (Dahlgren et al., 2016; CDC, 2013; Apperson et al., 2008). RMSF is the most commonly reported and the most severe tick-borne rickettsial illness in the U.S. (CDC, 2013; Apperson et al., 2008). Reported cases of all SFG rickettsioses have rapidly increased in the U.S. in recent years with 1.7 to 7 cases per million people from 2000 to 2007 (Openshaw at al. 2010; Kakumanu et al., 2016). Fortunately, during that same period there was a decrease in case fatality rates from 2.2% to 0.3% (Openshaw et al., 2010; Kakumanu et al., 2016). Changes in surveillance and diagnostic practices could be enhancing the reported incidence rates but the drop in case fatalities has not yet been clearly determined (Openshaw et al., 2010). Since medical treatment for tick-borne illnesses has not changed in the last few decades, other factors must be contributing to the perceived decrease in mortality.

Roughly 60% of all of the reported RMSF cases in the U.S. are found in five states: Oklahoma, Arkansas, North Carolina, Tennessee, and Missouri (CDC, 2010a; Openshaw et al., 2010; Hudman and Sargentini, 2016). In Missouri, as is the case in many other states, there are numerous tick vectors capable of transmitting pathogens but in some cases the vector competence is unclear (Hudman and Sargentini, 2016; Ostfeld, 2011; Hermance et al., 2014). Table 1 illustrates the total number of reported cases of RMSF, Lyme disease, ehrlichiosis, tularemia, and anaplasmosis during a six year period in Missouri. While many of the cases are confirmed for each of these diseases, there is often an inability to positively confirm the causative agent, most notably with RMSF. Positive confirmation typically relies on the detection of *R. rickettsii* or other spotted fever group DNA in a clinical specimen via amplification of a specific PCR target or the serological evidence of a fourfold change in IgG via Immunofluorescence Assay (IFA) between serum taken in the first week of illness and another one 2–4 weeks later (CDC, 2010a). Since reports are frequently based on only one lab result, positive confirmation is often not possible (Karen Yates, personal communication). In addition, since the tests use *R. rickettsii* or other SFG antibodies, rickettsiae that cross-react such as *R. montanensis, R. amblyommatis, R. parkeri*, or more than one rickettsiae could be responsible for the antibody titers rather than *R. rickettsii*, rendering the diagnosis of RMSF questionable.

Because some ambiguity about vector competence, pathogen virulence, vector range, etc., exists regarding tick-borne diseases in Missouri, a survey was carried out to assess local healthcare practitioners' awareness/opinions regarding the prevalence and diagnosis of

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Table 1
Confirmed and probable cases of Rocky Mountain spotted fever and other tick-borne diseases in Missouri, 2011–2016. Data provided by the Missouri Department of Health and Senior Services, Bureau of Reportable Disease Informatics.

TICK-BORNE DISEASE		2011	2012	2013	2014	2015	2016
Tularemia	Confirmed	8	17	19	12	10	20
	Probable	13	10	17	8	19	15
	Total	21	27	36	20	29	35
Rocky Mountain spotted fever	**Confirmed**	**13**	**4**	**2**	**2**	**4**	**4**
	Probable	**257**	**311**	**243**	**263**	**318**	**347**
	Total	**270**	**315**	**245**	**265**	**322**	**351**
Lyme disease	Confirmed	5	1	1	7	2	1
	Probable	3	1	2	3	3	9
	Total	8	2	3	10	5	10
Ehrlichiosis (All)	Confirmed	101	119	226	229	160	142
	Probable	93	109	172	163	102	98
	Total	194	228	398	392	262	240
Anaplasmosis (Anaplasma phagocytophilum)	Confirmed	1	0	1	0	1	1
	Probable	24	23	12	24	14	12
	Total	25	23	13	24	15	13

tick-borne diseases. In addition, ticks from two parks in St. Louis County in Eureka, Missouri, were evaluated to detect SFG rickettsiae, as well as to determine the most common tick species present, and if any other tick species has extended its range into the area.

2. Material and methods

2.1. Survey of healthcare practitioners

For the evaluation of local healthcare practitioners, a 14-question anonymous survey, which included the IRB consent form and a survey link, was sent to eighty-one St. Louis, MO, area hospital infectious diseases physicians and family practitioners (Appendix A in Supplemtary Materials). This was done during the months of October to December 2016. Questions about the prevalence and diagnosis of tick-borne diseases, diagnostic tests used, antibiotic selections and duration of use, and general tick and tick-borne diseases were included.

2.2. Tick collection and detection of Rickettsia spp

Collection of ticks was carried out by flagging through vegetation in Eureka, Missouri, at the Tyson Research Center (38°30′N, 90°32′W) and Lone Elk Park (38°31′N, 90°32′W) during the summers of 2015 and 2016 (May to September). The Tyson Research Center, a private 2000-acre field station operated by Washington University, is home to over 40 species of mammals and houses the Endangered Wolf Center. Lone Elk Park is a 546-acre county park adjacent to the Tyson Research Center, and it is home to the same species of mammals, but American bison and elk can also be found. These parks were chosen because of the high density of vertebrates in fence-enclosed areas and because they are located along the Mississippi Flyway of migratory birds, providing sources of potential hosts. They were also chosen because of the increase in reported tick-borne diseases contracted in rural areas of St. Louis County such as Eureka, Missouri, which corresponds to the increase in the state from 2011 to 2016 (Table 1).

Ticks were sorted into pools of a maximum ten individuals (with the exception of *Ixodes scapularis*) with respect to species and stage of development using a pictorial key (Keirans and Litwak, 1989) and screened by PCR (Smith et al., 2010) for *R.* spp., *R. rickettsii, R. montanensis,* and *R. amblyommatis* using species-specific primers (Table 2). DNA extractions for the different tick species were processed separately and all laboratory precautions were taken to prevent contamination during the analyses. To confirm the presence of the rickettsiae detected, *Rickettsia* spp.-specific *gltA* gene fragments (401 bp) were amplified in 50% of tick pools (Labruna et al., 2004). The confirmatory PCRs were replicated at Southern Illinois University Edwardsville and the University of Costa Rica with 100% overlap in results. The reaction products for 7 of the PCR-positive pools were sent to Macrogen (Korea) for sequencing; sequences were edited and compared to GenBank databases for species determination and confirmation.

3. Results and discussion

3.1. Survey of healthcare practitioners

The following results are based on the roughly 21% (17/81) response rate. Forty-two percent (7/17) of the physicians who responded had diagnosed and treated at least one or more patients with a tick-borne illness in the last five years. Many clinicians surveyed believed that Lyme disease (82%) and RMSF (58%) are the top two most commonly encountered tick-borne infections in Missouri. While this is true for RMSF, it is not the case for Lyme disease since it is reported at a much lower frequency than RMSF, ehrlichiosis, anaplasmosis, and tularemia (Table 1). In fact, there are uncertainties regarding whether Lyme disease exists in Missouri at all or if it is Southern Tick-Associated Rash Illness (STARI) (CDC, 2010a; Hudman and Sargentini, 2016). With regards to tick species, 9/17 (53%) associated their patients' tick bites with *Amblyomma americanum,* the lone star tick. Interestingly, 7/17 clinicians reported that they suspected that *Amblyomma maculatum*, the Gulf Coast tick, had parasitized their patients. While the Gulf Coast tick has been shown to be expanding its range further north and west over the last several decades (Wright et al., 2011; Sonenshine, 2018), there have been unpublished reports of its existence in the area (Solny Adalsteinsson and Karen Yates, personal communication). However, it has not yet been positively confirmed by the CDC in the St. Louis, Missouri area (CDC, 2010b). Consequently, the likelihood of a patient being bitten by this species is low.

Approximately 80% of responders based their suspicion of tick-borne diseases on symptoms. However, symptoms alone are not adequate for diagnosis since they could potentially resemble a multitude of other infections. Many physicians (14/17) ordered enzyme-linked immunosorbent assay (ELISA) to test for the presence of tick-borne infections. Current CDC recommendations propose an initial two-step laboratory test consisting of an ELISA and IFA (CDC, 2016) for Lyme disease. If positive, clinicians then have to order a Western Blot (WB) for IgG to determine a more probable diagnosis. The serological test of choice for tularemia is microagglutination, for ehrlichiosis/anaplasmosis is indirect immunofluorescence, and for rickettsiosis is micro-immunofluorescence (CDC, 2011). Hence, Lyme disease was suspected in a large number of cases with the physicians polled. Another clinically relevant aspect relates to the selection, initiation, and duration of a drug regimen. Seventy percent (12/17) of the responders reported doxycycline as the first choice for these tick-borne infections, and one clinician surveyed started empiric antibiotic therapy upon suspected tick-borne infection. But there was wide variability in the duration of treatment (1–5, 7–14, 15–21 days). CDC recommends no more or less than 14–21 days of antibiotic use (CDC, 2016). Nearly half (8/17) of the clinicians reported shorter (1–5 days) or longer (over 21 days) periods of treatment for their patients although the shorter regimen may reflect prophylactic treatment.

3.2. Tick collection and detection of Rickettsia spp

A total of 270 lone star ticks, *A. americanum* (Linneaus), 44 American dog ticks, *Dermacentor variabilis* (Say), and 2 blacklegged ticks, *Ixodes scapularis* (Say), were analyzed by PCR in this study to detect the presence of rickettsial species.

Eighty-nine percent (24/27 pools) of *A. americanum* pools were positive for *Rickettsia amblyommatis* but all were negative for *R. montanensis* and *R. rickettsii*. All seven pools of *D. variabilis* were positive for *R. amblyommatis*, 23% were positive for *R. montanensis*, and all were