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Geospatial Analysis of Rickettsial Species in Arkansas

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Cover Page Footnote
This research was made possible through funding from the Arkansas Biosciences Institute, Arkansas Division of Agriculture, and the Arkansas Department of Health. This project would not have been possible without the help of Arkansas Cooperative Extension and local Arkansans that submitted ticks. Analysis of data was assisted by the Center for Advanced Spatial Technologies at the University of Arkansas.

Geospatial analysis of rickettsial species in Arkansas

Meet the Student-Author

Amy Frank

Research at a Glance

- As tick-borne illnesses become more prevalent on a state and national level, Arkansas counties are in desperate need of risk assessment for Spotted Fever group *Rickettsia*.
- A portion of the Arkansas tick population was sampled and 34% of ticks were determined to be carriers of one or more disease-causing rickettsial species.
- Several counties in Arkansas face a significant exposure risk to Spotted Fever group *Rickettsia*, and varied sample size caused an incomplete picture to be formed of others.

I was raised in Greenwood, Arkansas and have spent most of my life surrounded by animals which led me to my passion for veterinary medicine. Following graduation from the University of Arkansas, I will begin veterinary school at Oklahoma State University. I have been very active in the Bumpers Honors Student Board serving as the Director of Student Relations, Secretary, and Chair. I was also an active member of the Representing, Educating, and Promoting Scholars team for the Department of Animal Science. During my senior year, I became involved with Student Organization Outreach and Involvement Experience serving as the Director of Administration. My experiences at the University of Arkansas were made remarkable by a long list of individuals. I would specifically like to thank my thesis advisor, Dr. Ashley Dowling, who was always available to answer questions. My family (present and future) supported and encouraged me daily. Lastly, I want to thank my fiancé. His love, support, and encouragement allow me to pursue my dreams. There are no words to accurately describe the gratitude I have for all he has done for me, but I hope to show him in the years to come.

Amy is pictured here receiving the Senior of Significance distinction from Chancellor Steinmetz (left) and Brandy Cox (right), Associate Vice Chancellor and Executive Director for Arkansas Alumni Association.

Geospatial analysis of rickettsial species in Arkansas

Amy Frank and Ashley Dowling†*

Abstract

Rickettsia species are obligate intracellular, arthropod-borne bacteria with the potential to cause multiple diseases including Rocky Mountain spotted fever (RMSF). Fleas, mites, and ticks serve as vectors for *Rickettsia*, but ticks are the primary vector of interest. Rocky Mountain spotted fever and other rickettsial diseases have continued to gain importance in both human and veterinary medicine as RMSF is the most common tick-borne disease within the United States according to the Lyme and Tick-Borne Disease Research Center. A statewide citizen science project was utilized to determine the prevalence of Spotted Fever Group (SFG) *Rickettsia* in Arkansas. This project yielded results in 64 of Arkansas' 75 counties. Results were utilized to determine prevalence in each of the represented counties and then compiled into a geospatial representation of the data. It was determined that 34.32% of the ticks sampled were carriers of one or more rickettsial species. As the samples were divided by county, multiple counties were shown to have a concerningly high exposure risk for SFG *Rickettsia*. There were six species of ticks represented throughout this study with *Amblyomma americanum* being the most common. There were also six species of SFG *Rickettsia* found within the samples. The small portion of ticks that underwent further analysis to determine the specific rickettsial species present indicated that *Rickettsia amblyommatis* is likely the most common SFG *Rickettsia* in Arkansas.

* Amy Frank is a May 2019 honors program graduate with a major in Animal Science with a pre-professional concentration.
† Ashley Dowling, the faculty mentor, is an associate professor in the Department of Entomology.

Introduction

Rickettsia are bacteria that live and proliferate within the cells of host organisms and have the potential to cause diseases in humans such as Rocky Mountain Spotted Fever (RMSF) (Paddock et al., 2004). Ticks operate as the primary vector of *Rickettsia* species allowing for the spread of potentially fatal diseases in humans and various animal species (Walker, 1996). Human patients endure nonspecific symptoms including fever, gastrointestinal upset, and headaches but more serious symptoms can progress such as severe myalgia, photophobia, and focal neurologic deficits (CDC, 2017a). In canines, rickettsial organisms attack vascular endothelial cells resulting in severe vasculitis, fever, ocular lesions, neurologic dysfunction, and edema (Low and Holm, 2005). Affected individuals report history of a tick bite in only 55% to 60% of cases and estimates show 60% to 75% of people are incorrectly diagnosed at the initial physician visit (Biggs et al., 2016; Herrman et al., 2014). A misdiagnosis can have severe consequences due to advanced pathological changes occurring (Raghavan et al., 2016; Gasser, 2001; Mayo Foundation, 2018).

Rickettsial organisms are typically divided into two groups, the typhus group (TG) and the spotted fever group (SFG), based primarily on distribution, pathogenicity, clinical presentation, immunological reactivity, DNA G +C mol% content and intracellular position (Fournier et al., 1998; Scarpulla et al., 2016; Eremeeva et al., 2006). In 2010, the Council of State and Territorial Epidemiologists made a push for Rocky Mountain spotted fever (RMSF) being reported under the SFG in an attempt to facilitate more complete local and national reporting (Council of State and Territorial Epidemiologists, 2009). State health departments, including Arkansas, have recently made a push for increasing submission rates and raising awareness for tick-borne diseases (Raghavan et al., 2016).

Concern about vector-borne diseases in pets is evident by the expanding use of ectoparasite preventatives (Bowman et al., 2009). In 2003, more than half of pet owners in the United States reported using parasite preventatives (Bowman et al., 2009). The rickettsial species capable of affecting humans and canines are found to be homologous, and studies (Herrman et al., 2014) have cited canines as potential reservoirs for tick-borne diseases (Herrman et al., 2014; Warner and Marsh, 2002; Paddock et al., 2002; Kidd et al., 2006). A 40-state study found Arkansas to have the second-highest level of tick-infested canines with the six border states falling within the top ten (Raghavan et al., 2007). The risk of exposure and contraction varies in different regions with North Carolina, Oklahoma, Arkansas, Tennessee, and Missouri contributing to over 60% of RMSF cases (Atkinson et al., 2012; CDC, 2017a).

While data are readily available regarding the number of human cases involving *Rickettsia*, there is limited data demonstrating the prevalence. Rickettsial species have increased their role in animal and human health during the last few decades, which makes the need for further data apparent (Bowman et al., 2009). A geospatial analysis showing the prevalence of *Rickettsia* in Arkansas ticks may place Spotted Fever Group *Rickettsia (*SFGR) at the forefront of physicians' and veterinarians' minds. The analysis can demonstrate the areas of Arkansas that are at the greatest risk for spreading SFGR, so individuals will also be aware of the disease and the risk for contraction.

Materials and Methods

Tick Collection

In order to obtain ticks from across the state, local Arkansans were recruited to participate in the sampling process through a citizen science project. Collection kits containing five color-coded vials containing 95% ethanol and a locality recording card were distributed to all 75 Arkansas county extension offices and handed out by county extension agents. When residents collected the specimens, they were asked to record locality information or GPS coordinates. After completing the kit, citizens mailed the ticks to the Department of Entomology at the University of Arkansas or returned the tick kits to their county extension office for delivery to the University. Kits were also supplied to veterinary and medical clinics around the state. The ticks were then identified and recorded into the project database.

Molecular Methods

The DNA was extracted from individual adult tick specimens using Invitrogen™ PureLink™ Genomic DNA Mini Kits (Invitrogen, Carlsbad, Calif.) following the instructions contained therein. Nymphal ticks from the same collection event were pooled (up to five individuals per pool) and then extracted using the same Invitrogen kit. Whole ticks were extracted intact, without cutting or crushing before extraction as this was determined to not affect the extraction efficiency. The DNA extracts were screened for the presence of rickettsial species via traditional polymerase chain reaction (PCR). Fragments of the 17-kDa antigen gene were targeted using primers specific to the spotted fever and typhus group *Rickettsia* (Rr17k.1p & Rr17k.539n from Ishikura et al., 2002). Resulting products were visualized on a 1x agarose gel and a subset of positive samples was purified using Invitrogen PureLink PCR Purification kits following instructions therein. Purified samples were sent to Macrogen USA (Macrogen Corp., Rockville, Md.) for sequencing using the same PCR primers. Raw sequences were confirmed through a comparison of existing sequence data in the national sequence repository GenBank.

Data Analysis

Data analysis was conducted through Aeronautical Reconnaissance Coverage Geographic Information Systems (ArcGIS; Esri, Redlands, Calif.). This system allowed for storage, manipulation, and visualization of data with the purpose of displaying or analyzing information about places or events. The analysis was conducted in collaboration with the University of Arkansas Center for Advanced Spatial Technologies (CAST). Due to the sampling technique used with the project, prevalence is the best determinant of SFGR distribution. It helps filter out the discrepancies caused by over or under-representation of regions. The positive result prevalence for each of the 75 counties was determined using ArcGIS. The prevalence was then displayed as a geographic heat map based on obtained levels of significance. Geovisualization displays geospatial information in an interactive manner which allows for conclusions to be made and spatial patterns to be revealed.

Results and Discussion

Over the course of the study, 4676 ticks were obtained from Arkansas counties and analyzed for the presence of rickettsial pathogens (Fig. 1). Of the analyzed specimens, 1605 ticks were found to be positive (Fig. 2) with the remaining 3070 ticks being negative for SFGR. Results were grouped and evaluated by county with samples being obtained from 64 of Arkansas's 75 counties. Prevalence of rickettsial species was determined using the following calculation:

$$\frac{Specimens\ positive\ for\ SFGR}{Total\ specimens\ screened} = Prevalence\ of\ SFGR$$

(Fig. 3). During specimen analysis, several characteristics were recorded such as the species of tick, sex, and life stage (Table 1). Prevalence by tick species in regard to the presence of SFGR was also observed and recorded (Table 2). There were 233 ticks that underwent a closer analysis to determine the specific *Rickettsia* specie(s) that was present (Table 3). The following *Rickettsia* species were found to be present in sampled ticks: *R. montanensis, R. amblyommatis, R. andeanae, R. bellii, R. rickettsii*, and *R. raoultii*.

The goal of this study was to determine the largest risk areas within Arkansas for a person or animal to become exposed to Spotted Fever Group *Rickettsia*. This is one of the only existing studies conducted utilizing geospatial analysis techniques to determine the geographic distri-

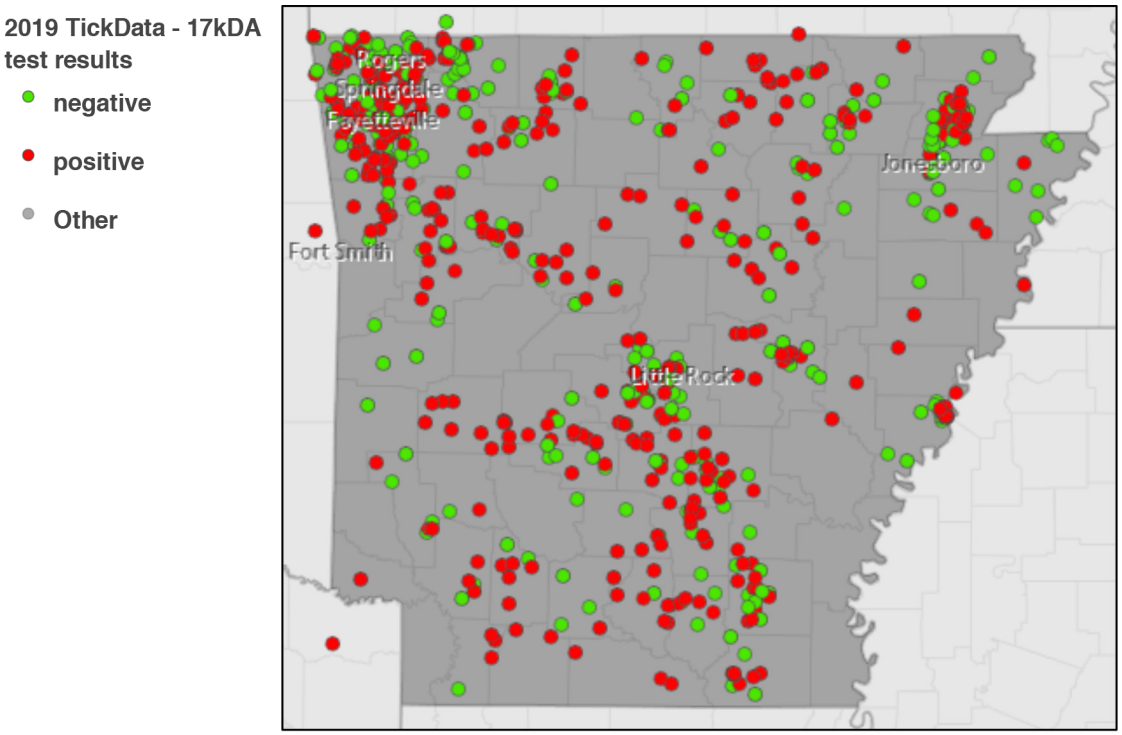

Fig. 1. Map of Arkansas displaying where tick samples were obtained based on locations given by citizen participants. Symbols represent the presence or lack of Spotted Fever Group *Rickettsia*.

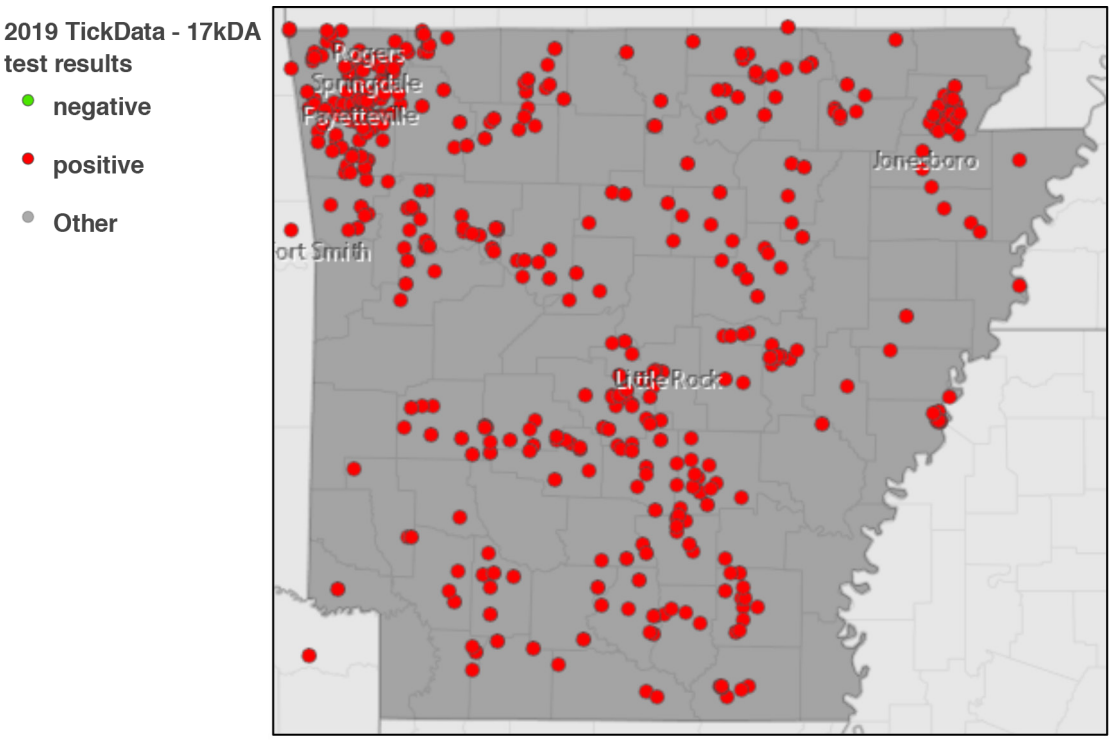

Fig. 2. Map of Arkansas displaying where tick samples were obtained that were found to be positive for the presence of Spotted Fever Group *Rickettsia*.

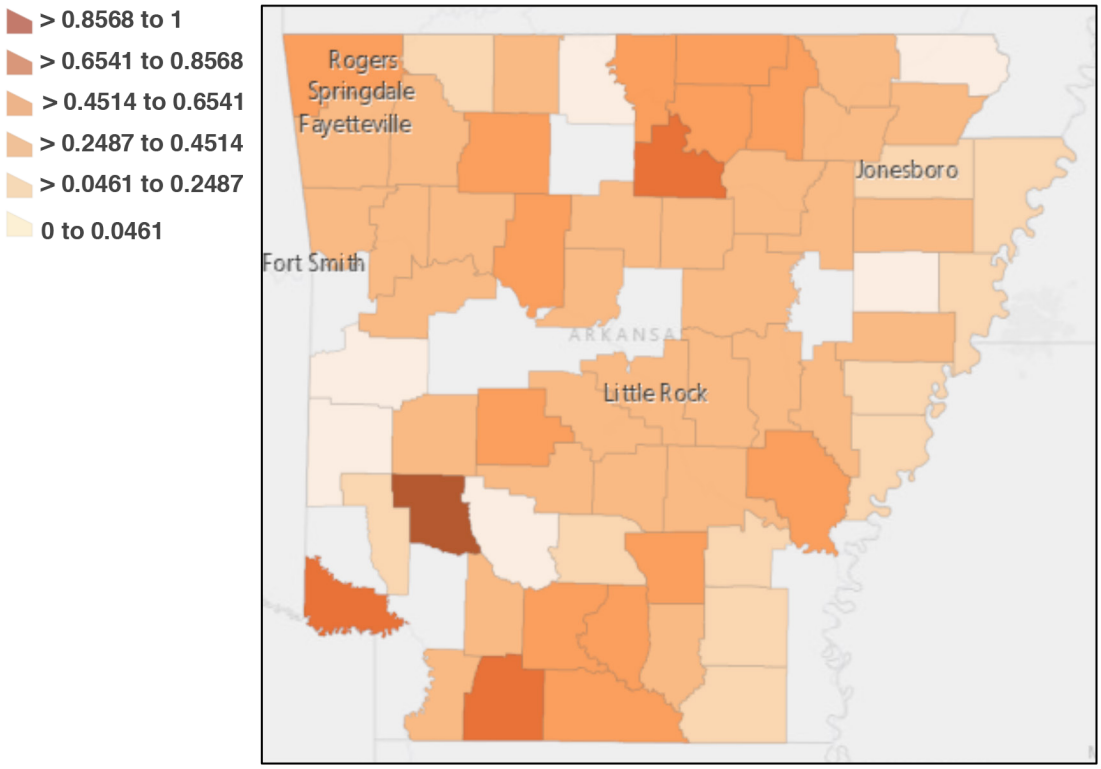

Fig. 3. Map of Arkansas displaying where tick samples were obtained that were found to be positive for the presence of Spotted Fever Group *Rickettsia*.

bution for SFGR in Arkansas, and therefore the areas that pose the greatest threat to human and animal health in the state. Samples were grouped based on which county in Arkansas they originated. This is because health departments tend to divide disease risk based on county.

When utilizing geospatial analysis, it is important to be aware of the modifiable areal unit problem (MAUP). This problem is a statistical biasing effect that occurs when samples are used to represent information for an area (Altaweel, 2018). The area is based on arbitrary boundaries, and therefore the analysis is inconsistent with real-world data. This is a common issue with health spatial statistics since statistics are typically reflecting spatial factors specific for that disease or the needs of the study (Altaweel, 2018). In this study, the prevalence of SFGR was applied to a map to demonstrate the risk of disease. The prevalence was grouped by county meaning that this study does technically fall under the criteria of the MAUP problem. To counteract the effect, more evaluation would need to be done using multiple random parameter settings. That would be irrelevant for this study, as the goal is to make the information accessible and usable to local health departments.

When looking at the display of SFGR prevalence in Arkansas (Fig. 3), it is evident that there are regions of Arkansas that face a greater risk than others. This study determined that 34.32% of the ticks sampled were carriers of SFGR. In order to determine the areas with the greatest associated risk, prevalence was utilized. This is to accommodate for the vast differences in sample size. The range in sample size was 0 to 1119 ticks.

Part of the testing process for the specimens was to determine species, sex, and life stage. The species of the tick is of interest because it is important to know which species make up the tick population of Arkansas. It is also essential to know which tick species are acting as reservoirs for SFGR. *Amblyomma americanum* was found to be the most common species making up 71% (3338 individuals) of the total ticks collected throughout this experiment. When *A. americanum* was tested for the presence of SFG *Rickettsia*, 1414 ticks demonstrated positive results. This translates to 42% of the *A. americanum* ticks tested being found to contain *Rickettsia*. Each of the six tick species found throughout this study is considered capable of transmitting rickettsial species (Lee et al., 2018; Levin et al., 2017). *Ixodes scapularis* was found to have the highest

Table 1. Species of ticks with associated common names and prevalence.

Tick species	Common name	No. ticks screened	Percentage of total[a]
A. americanum	Lone Star Tick	3338	71.39%
A. maculatum	Gulf Coast Tick	151	3.23%
D. variabilis	American Dog Tick	943	20.17%
D. albipictus	Winter Tick	1	0.02%
I. scapularis	Blacklegged Tick	59	1.26%
R. sanguineus	Brown Dog Tick	184	3.93%
Total		4676	

[a] Determined with the following calculation: $\frac{No.\ Ticks\ Screened}{4676}\ x\ 100.$

Table 2. Species of ticks with associated common names and prevalence of Spotted Fever Group *Rickettsia* (SFGR).

Tick species	Common name	No. ticks positive for SFGR	Percentage positive for SFGR[a]
A. americanum	Lone Star Tick	1414	42.36%
A. maculatum	Gulf Coast Tick	54	35.76%
D. variabilis	American Dog Tick	96	10.18%
D. albipictus	Winter Tick	0	0.00%
I. scapularis	Blacklegged Tick	41	69.49%
R. sanguineus	Brown Dog Tick	0	0.00%
Totals		1605	34.32%

[a] Determined with the following calculation: $\frac{No.\ Ticks\ Positive\ for\ SFGR}{No.Ticks\ Screened}\ x\ 100.$

percentage of ticks positive for SFGR. While *I. scapularis* was found to be less than 2% of the tick population, 69% were found to carry SFG *Rickettsia* species.

A small portion of the ticks sampled was randomly selected to undergo further analysis to determine the actual member of the SFG *Rickettsia* that was present. There were six *Rickettsia* species found to be present in Arkansas ticks. Interestingly, *R. raoultii* was found in 12 ticks, but only in the presence of *R. montanensis*. This is not considered uncommon as *R. raoultii* has been found to have near relationships with other members of SFGR (Li et al., 2018). Of the rickettsial pathogens found in samples, *R. montanensis* and *R. bellii* are considered of less significance as their capability to transmit disease has yet to be proven, but research has begun to suggest that *R. bellii* could eventually be found to be disease causing (Mullen and Durden, 2009; Parola et al., 2014). *R. amblyommatis, R. andeanae, R. rickettsia*, and *R. raoultii* are known to be disease-causing members of the SFGR (Apperson et al., 2008; Delgado-de la Mora et al., 2019; Mullen and Durden, 2009). This is concerning information since the most common pathogen, *R. amblyommatis*, was found in the most common tick, *A. americanum*.

In order to obtain a better understanding of SFGR in Arkansas, sample sizes would need to be increased for each of the counties in Arkansas. The counties that did not respond to the study or responded in low numbers should be specifically targeted. While the prevalence varied drastically from county to county, the potential to be exposed to SFG *Rickettsia* species was abundantly clear. Other information that could be utilized in this study is the proportion of male to female ticks in the population as well as the proportion of the various life stages. This information could be useful in investigating the implication that rickettsial species have on their host. Some species of the SFG *Rickettsia* are known to have lethal effects on their tick hosts (Niebylski et al., 1999). Furthermore, time of year the specimen is obtained could be relevant information regarding when humans and animals are at most risk for being exposed to ticks.

Conclusions

Understanding the distribution of SFGR in Arkansas is essential to the veterinary and human health fields. This study showed evident regions of Arkansas that present a greater SFGR exposure risk than others. The Arkansas tick population that was sampled displayed that 34.32% of ticks are carriers of one or more rickettsial species. The aggressive human-biting tick, *A. americanum*, was the most prevalent species in the sampled population and displayed a SFGR prevalence of 42%. Concerningly *I. scapularis* was found to be a small portion of the population but showed a remarkably high SFGR prevalence. All tick species obtained throughout this project are confirmed vectors of SFGR which demonstrates why Arkansas has repeatedly been found to have one of the highest incidences of SFGR.

Table 3. Species of Spotted Fever Group *Rickettsia* (SFGR) found in ticks sampled[a].

	Known disease-causing SFGR			
Tick species	***R. amblyommatis***	***R. andeanae***	***R. rickettsii***	***R. raoultii*[b]**
A. americanum	206	0	0	0
A. maculatum	0	0	1	0
D. variabilis	0	1	0	12
D. albipictus	0	0	0	0
I. scapularis	0	0	0	0
R. sanguineus	0	0	0	0

	Not known disease-causing SFGR	
	R. bellii	***R. montanensis***
A. americanum	0	0
A. maculatum	0	0
D. variabilis	1	19
D. albipictus	0	0
I. scapularis	0	0
R. sanguineus	0	0

[a] There were a total of 233 ticks that underwent further analysis to determine which member(s) of the SFGR was present with some ticks representing more than one SFGR member.
[b] This species was only found in the presence of *R. montanensis*.
Note: The ability to cause disease was based on information found in "Update on tick-borne rickettsioses around the world: A geographic approach" by Parola et al., 2014. American Society for Microbiology.

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This research was made possible through funding from the Arkansas Biosciences Institute, University of Arkansas System Division of Agriculture, and the Arkansas Department of Health. This project would not have been possible without the help of Arkansas Cooperative Extension and local Arkansans that submitted ticks. Analysis of data was assisted by the Center for Advanced Spatial Technologies at the University of Arkansas.

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