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Geospatial Analysis of Rickettsial Species

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Abstract

Rickettsia species are obligate intracellular, arthropod-borne bacteria with a 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. RMSF 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's 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 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 Spotted Fever Group *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.

Introduction

Rickettsia species are obligate intracellular, arthropod-borne bacteria with ticks, fleas, and mites serving as potential vectors (Paddock et al., 2004). *Rickettsia* has the potential to cause Rocky Mountain spotted fever (RMSF) (Walker, 1996). This potentially fatal disease is tick-vectored and occurs in humans and various animal species. Affected individuals report history of a tick bite in only 55-60% of recorded cases (Biggs et al., 2016). 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 which can result in severe vasculitis, fever, ocular lesions, neurologic dysfunction, and edema (Low & Holm, 2005).

According to the Lyme and Tick-Borne Diseases Research Center, RMSF remains the most common tick-borne disease in the United States with 2-5% of patients dying from the infection (Low & Holm, 2005). Individuals that develop RMSF are likely to avoid complications if treated within the first five days of symptom onset, but physicians often struggle to diagnose RMSF quickly since the symptoms mirror many other diseases (Mayo, 2018). The Centers for Disease Control and Prevention (CDC) reported that RMSF incidence increased from less than 2 cases per million persons in 2000 to over 8 cases per million persons in 2008 (Atkinson et al., 2012). Hospitalization rates have decreased since the 1980s, but almost 25% of patients still required hospitalization in 2014 (CDC, 2017c). The most important factors in reducing negative outcomes of RMSF are minimized tick exposure, prompt removal of ticks, and early diagnosis with proper treatment (Warner & Marsh, 2002).

The number of species included in *Rickettsia* is expanding, and can be characterized by microbiological characteristics, distribution, ecology, pathogenicity, and association with arthropod hosts (Scarpulla et al., 2016; Eremeeva et al., 2006). Rickettsial organisms are typically divided into two groups, the typhus group (TG) and the spotted fever group (SFG), based primarily on clinical presentation, immunological reactivity, DNA G +C mol% content and intracellular position (Fournier et al., 1998). In 2010, the Council of State and Territorial Epidemiologist made a push for Rocky Mountain spotted fever being reported under the name Spotted Fever Group *Rickettsia* (SFGR) in an attempt to facilitate more complete local and national reporting (Council of State and Territorial Epidemiologists, 2009). RMSF is only one of many diseases caused by SFGR, other diseases include Rickettsialpox, Cat flea rickettsiosis, Maculatum infection, Tidewater spotted fever, and American boutonneuse fever.

Ticks have the potential to interact with different vertebrate species, which allows them the opportunity to be efficient disease vectors by acquiring a variety of organisms present in the blood of their hosts (Munderloch, 2011). Concern about vector-borne diseases in pets is evident by the expanding use of tick, mosquito, and heartworm preventatives in veterinary medicine (Bowman et al., 2009). In 2003, more than half of pet owners in the United States reported using parasite preventatives (Bowman et al., 2009). Acaricides and insecticides have not been proven as a sole effective preventative for breaking enzootic transmission cycles (Bowman et al., 2009). The rickettsial species capable of affecting humans and canines are found to be homologous, and (Herrman et al., 2014) studies have cited canines as potential reservoirs for tick-borne diseases (Herrman et al., 2014; Warner & Marsh, 2002; Paddock et al., 2002; Kidd et al., 2006).

Dermacentor andersoni was incriminated as the first vector of RMSF by H.T. Ricketts, but species such as *Hemaphysalis leporispalustris, D. parumapertus, D. variabilis, D.*

occidentalis, Rhipicephalus sanguineus, Amblyomma maculatum, A. americanum, Ixodes dentatus, I. pacificus, I. cookei, I. brunneus, and I. texanus have since been found to be infected with rickettsial bacteria (Munderloch, 2011; Raoult & Roux, 1997). The United States has multiple tick species that have been confirmed vectors of SFGR including *D. variabilis, D. andersoni* and *R. sanguineus*. The spatial range of these ticks has changed in the past decade (Raghavan et al., 2016).

Spotted Fever Group *Rickettsia* is not evenly distributed throughout the continental United States; therefore, the risk of exposure and contraction varies in different regions (Atkinson et al., 2012). Most species of ticks tend to thrive in wooded areas (CDC, 2017b) with warm temperatures and high humidity (Herrman et al., 2014). North Carolina, Oklahoma, Arkansas, Tennessee, and Missouri contribute over 60% of RMSF cases (CDC, 2017a). An area in Eastern Arizona experienced a high transmission rate between 2003 and 2014 with a case-fatality rate of almost 10% (CDC, 2017c). The CDC speculated the large number of free-roaming dogs present in affected communities were at fault for the disease's rapid spread (CDC, 2017c). It is believed that veterinary professionals should warn pet owners of the increased risk of tick-borne diseases for both the owner and pet's sake (Nicholson et al., 2010). Before veterinarians are capable of properly warning pet owners of the dangers associated with ticks, veterinarians must first be aware of the likelihood their patient will be in contact with disease causing pathogens such as SFGR.

While data is readily available regarding 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 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.

Purpose & Objectives

The purpose of this study was to describe the spatial distribution of the obligate intracellular *Rickettsia* species in Arkansas through traditional PCR testing of ticks. To meet this purpose, the following objectives were created:

1. What is the distribution range of Spotted Fever Group *Rickettsia* in Arkansas?
2. Are there any high-risk areas within?
3. Are previously published associations between cases and environmental conditions reflected in this spatial distribution of *Rickettsia* species in Arkansas?

Review of Literature

RMSF has appeared in literature since 1899 when it was described as "an acute endemic, noncontagious, but probably infectious, febrile disease, characterized clinically by a continuous moderately high fever, severe arthritic and muscular pains, and a profuse petechial or purpural eruption in the skin, appearing first on the ankles, wrists, and forehead, but rapidly spreading to all parts of the body" (Maxey, 1899). RMSF is the most commonly reported tickborne rickettsial disease in the United States and is caused by *Rickettsia rickettsii* (Dantas-Torres, 2007). As a tick feeds, an interaction with the host's immune system allows for a pathogen to be transmitted as the immune system is suppressed and becomes immunologically inactive (Munderloch, 2011). Symptoms of RMSF include anorexia, lethargy, fever, thrombocytopenia, leukocytosis, and

hypoproteinemia (Gasser, 2001). Since symptoms are nonspecific and develop a few days into infection, initial diagnosis is often incorrect (Herrman et al., 2014; Raghavan et al., 2016). Estimates show 60-75% of human cases are incorrectly diagnosed at the initial physician visit (Herrman et al., 2014). A misdiagnosis can have severe consequences in human and veterinary medicine because delay of treatment allows for advanced pathological changes to occur (Gasser, 2001). Tetracyclines have offered the greatest clinical treatment results, with doxycycline being the drug of choice for physicians and veterinarians (Openshaw et al., 2010). Delay in antibiotics increases the probability of a fatal outcome (Raghavan et al., 2016; Gasser, 2001). Human fatality rates have been on the decline during the last decade, but the incidences of RMSF have increased in the United States (Openshaw et al., 2010; Paddock et al., 2004). Canine patients, if treated properly in the early stages of RMSF, typically avoid irreversible damage and mortality (Gasser, 2001).

Rocky Mountain spotted fever has been reported in most of the continental United States, but incidence is higher in the lower Midwest and southern US (Raghavan et al., 2016). Oklahoma, Arkansas, Missouri, Tennessee, North Carolina, and South Carolina have demonstrated higher case numbers in recent years (Atkinson et al., 2012; Raghavan et al., 2016). 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). Arthropod life-cycles are highly dependent on climatic conditions with warm conditions and high humidity being the most favorable conditions for ticks (Herrman et al., 2014; Raghavan et al., 2016). Peak tick activity is associated with summer months and has allowed for the prediction of RMSF seasonal trends (Openshaw et al., 2010). Shifts in demographics and human population has forced dramatic changes to distribution of natural habitats and resources, which has caused new and increased

interaction between people and animals (Munderloch, 2011). This increases transmission opportunities for zoonotic diseases. In previous years, surveillance procedures for tick-borne diseases like RMSF have utilized a passive system (Openshaw et al., 2010). Beginning in 2010, the CDC adopted the definition of Spotted Fever (SFG) Rickettsiosis for reporting *R. rickettsii* as well as other *rickettsial* species, such as *R. parkeri* and *R. amblyommatis.* This would facilitate more complete reporting conditions associate with SFGR (Council of State and Territorial Epidemiologists, 2009). State health departments, including Arkansas and Oklahoma, have recently made a push for increasing submission rates and raising awareness for tick-borne diseases (Raghavan et al., 2016). Federal funds have also been increasingly allocated to support state programs which allows for a strengthened surveillance of tick-borne diseases (Openshaw et al., 2010). The increased awareness of tick-vectored diseases has influenced individuals to ask for specialized laboratory testing to determine if the ticks found in homes, on people, or pets are infected with a potentially disease causing pathogen (Scarpulla et al., 2016). While it is important to mention that the detection of a pathogen in a tick does not guarantee transmission to an individual, SFG Rickettsiosis should be included in differential diagnoses for patients in endemic regions (Scarpulla et al., 2016; Gasser, 2001).

Molecular tools and analytical techniques have enabled scientists to gain a better understanding of SFGR (Munderloch, 2011). Polymerase chain reaction (PCR) and rapid sequencing techniques for DNA became available in the 1980s, which allowed for phylogenetic inferences to be made more efficiently (Fournier et al., 1998). Since then, bacterial taxonomy of *Rickettsia* has been based on the comparative analysis of gene sequencing following amplification by PCR (Xu & Raoult, 1998). Detection of SFG *Rickettsia* using PCR-based methods offers researchers the ability to circumvent the need for culture while allowing for more

sensitive and specific alternatives (Raoult & Roux, 1997). PCR amplification can be performed with a variety of samples including arthropod tissues, blood, and skin biopsy samples if the sample are collected prior to initiation of antibiotic treatment and before antibodies are detectable (Raoult & Roux, 1997). Throughout the early 2000's, the most common diagnostic method utilized for canine cases of rickettsial disease was PCR on serum, but sensitive and highly specific ELISA diagnostic kits have since been introduced into veterinary medicine (Herrman et al., 2014). These kits include the SNAP 3Dx (introduced in 2001) and SNAP 4Dx (introduced in 2006) tests by IDEXX Laboratories (Herrman et al., 2014). Tests such as these increased diagnostic testing in veterinary practices due to the ease and affordability associated with in-house testing. Changes in diagnostic methods and accuracy could have influenced the recent increase in case reporting allowing for more accurate assessments to the spatial range of rickettsial diseases (Raghavan et al., 2016).

The presence of rickettsial pathogens has been evaluated in numerous studies around the world using a variety of techniques. Comparative analyses have been conducted using comparative sequences of the 16S rRNA-encoding gene, the citrate synthase gene (gltA), 17-kDa antigen gene (htrA), or the outer membrane protein A (rOmpA)-encoding gene (ompA) and outer membrane protein B (rOmpB)-encoding gene (ompB) (Xu & Raoult, 1998; Raoult & Roux, 1997). These PCR assays are not specific for individual rickettsial species, but further analysis can be conducted to discern species (Raoult & Roux, 1997).

Methods

To obtain the required data, Arkansas residents were asked to collect ticks from their local areas and send them to the University of Arkansas Entomology Department. The ticks were screened for the presence of rickettsial species via traditional polymerase chain reaction (PCR).

Fragments of the 17-kDa antigen gene were selected for detection since this gene is *Rickettsia* genus-specific (Blair et al., 2004). When residents collected the specimens, they were asked to record locality information or GPS coordinates. The location in which affected ticks originated from was utilized to construct a geospatial analysis of SFGR throughout Arkansas.

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. 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

DNA was extracted from individual adult tick specimens using Invitrogen PureLink Genomic DNA Mini Kits 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. 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., 2003). Resulting products were visualized on a 1x agarose gel and a subset of positive samples were purified

using Invitrogen PureLink PCR Purification kits following instructions therein. Purified samples were sent to Macrogen USA for sequencing using the same PCR primers. Sequence contigs were assembled using DNA Baser and spotted fever group *Rickettsia* identity was confirmed through a BLAST search in GenBank.

Data Analysis

Data analysis is conducted through Aeronautical Reconnaissance Coverage Geographic Information Systems (ArcGIS). This system allows for storage, manipulation, and visualization of data with the purpose of displaying or analyzing information about places or events. The analysis was conducted in collaborations 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. 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

Over the course of the study, 4676 ticks were obtained from Arkansas counties and analyzed for the presence of rickettsial pathogens (Figure 1). Of the analyzed specimens, 1605 ticks were found to be positive (Figure 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 (Table 1). The 11 counties that were unresponsive to the study were Chicot, Desha, Faulkner, Hempstead, Miller, Perry, Searcy, Sebastian, Sevier, Woodruff, and Yell. Prevalence of rickettsial species was determined using the following calculation:

$\frac{Specimens\ Positive\ for\ SFGR}{Total\ Specimens\ Screened} = Prevalence\ of\ SFGR$ (Figure 3). During specimen analysis, several characteristics were recorded such as the species of tick, sex, and life stage (Table 2). Prevalence by tick species in regards to the presence of SFGR was also observed and recorded (Table 3). There were 233 ticks that underwent a closer analysis to determine the specific *Rickettsia* specie(s) that was present (Table 4). 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*. When *R. raoultii* was found to be present, it was always in the presence of *R. montanensis* in *D. variabilis* (Table 3). *R. amblyommatis* was the most common Rickettsial species found and was solely found in *A. americanum*.

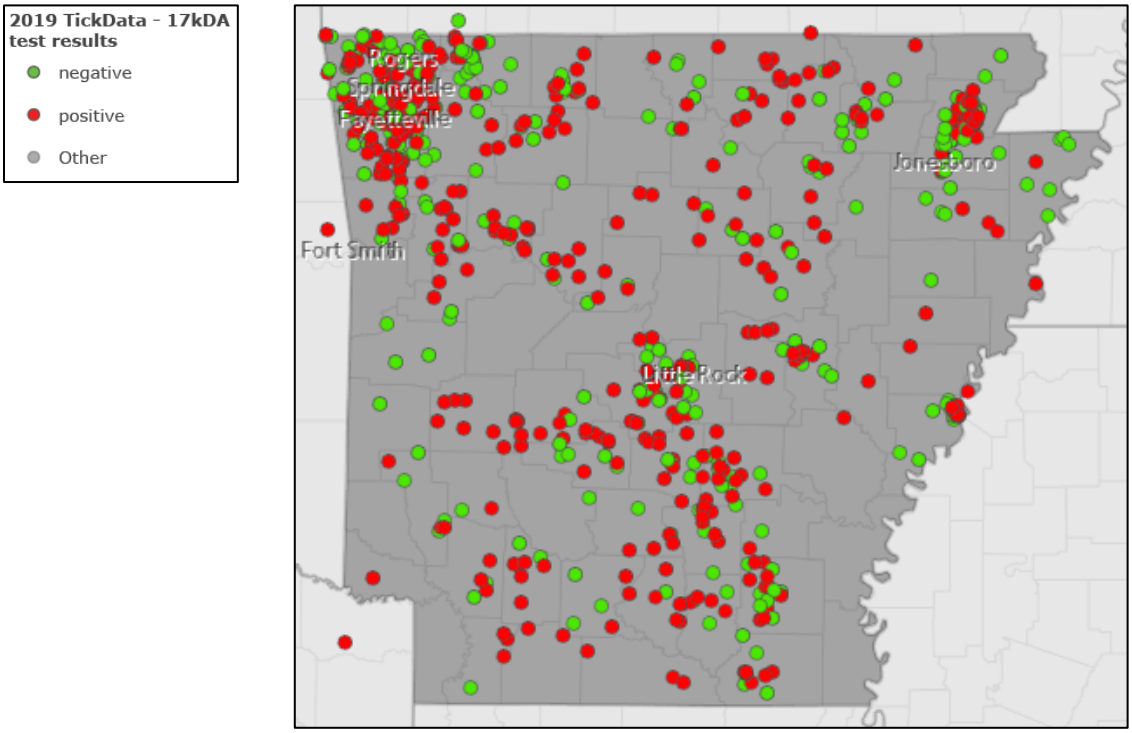

Figure 1. Map of Arkansas displaying where tick samples were obtained based on locations given by citizen participants. Symbols represent presence or lack of Spotted Fever Group *Rickettsia*.

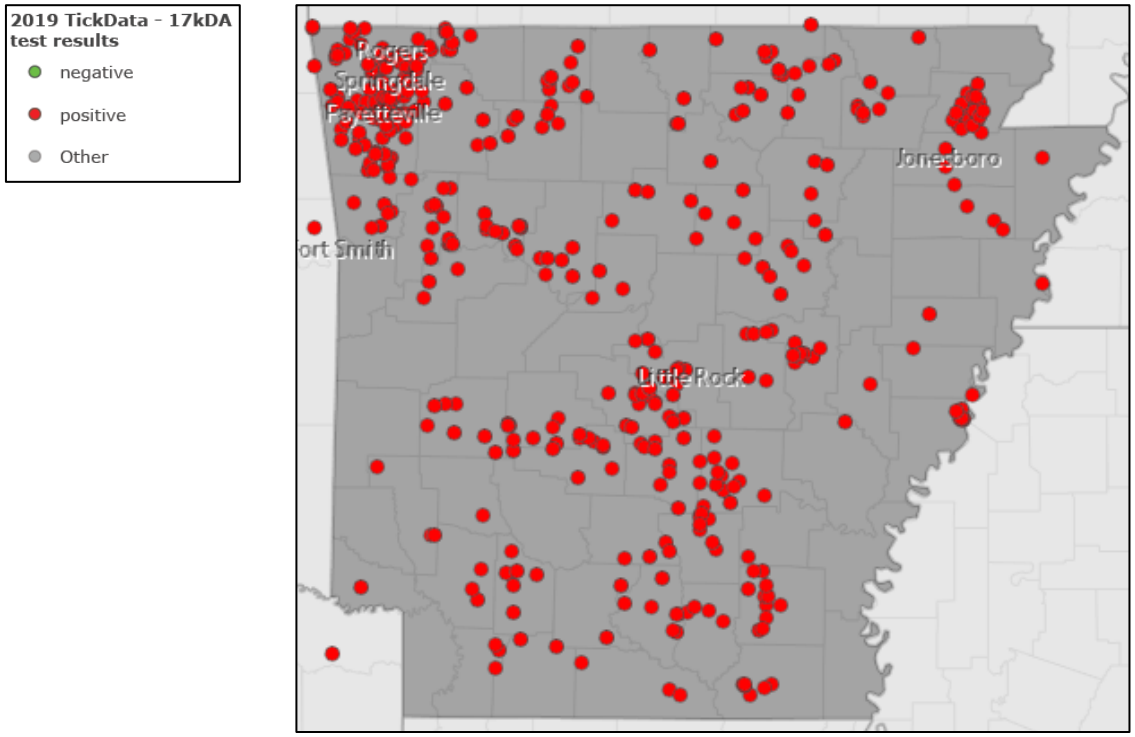

Figure 2. Map of Arkansas displaying where tick samples were obtained that were found to be positive for the presence of Spotted Fever Group *Rickettsia*.

Table 1.

Ticks Collected Within Arkansas Recorded by County of Origin

County	No. of Ticks Screened	No. of Positive Ticks	Prevalence of SFGR
Arkansas	13	7	0.539
Ashley	36	7	0.194
Baxter	48	22	0.458
Benton	344	100	0.291
Boone	67	23	0.343
Bradley	14	5	0.357
Calhoun	27	12	0.444
Carroll	151	46	0.305
Clark	2	0	0.000
Clay	2	0	0.000
Cleburne	21	8	0.381
Cleveland	27	13	0.482
Columbia	22	15	0.683
Conway	24	9	0.375
Craighead	34	3	0.088
Crawford	68	17	0.250
Crittenden	11	1	0.091
Cross	2	0	0.000

Dallas	54	10	0.185
Drew	71	16	0.225
Franklin	147	44	0.299
Fulton	81	44	0.543
Garland	40	20	0.500
Grant	56	16	0.286
Greene	320	87	0.272
Hot Spring	66	29	0.439
Howard	46	11	0.239
Independence	16	7	0.438
Izard	89	52	0.584
Jackson	106	44	0.415
Jefferson	77	25	0.325
Johnson	207	76	0.367
Lafayette	18	6	0.333
Lawrence	35	14	0.400
Lee	21	1	0.048
Lincoln	25	4	0.160
Little River	5	4	0.800
Logan	95	30	0.316
Lonoke	54	17	0.315
Madison	54	19	0.352
Marion	1	0	0.000
Mississippi	8	1	0.125
Monroe	4	1	0.250
Montgomery	125	37	0.296
Nevada	30	13	0.433
Newton	149	76	0.510
Ouachita	26	14	0.539
Phillips	54	13	0.241
Pike	1	1	1.000
Poinsett	12	4	0.333
Polk	5	0	0.000
Pope	62	30	0.484
Prairie	75	32	0.427
Pulaski	131	56	0.428
Randolph	23	9	0.391
St. Francis	8	3	0.375
Saline	77	21	0.273
Scott	24	0	0.000
Sharp	65	35	0.539
Stone	23	17	0.739
Union	4	2	0.500
Van Buren	24	9	0.375
Washington	1119	355	0.317
White	30	12	0.400

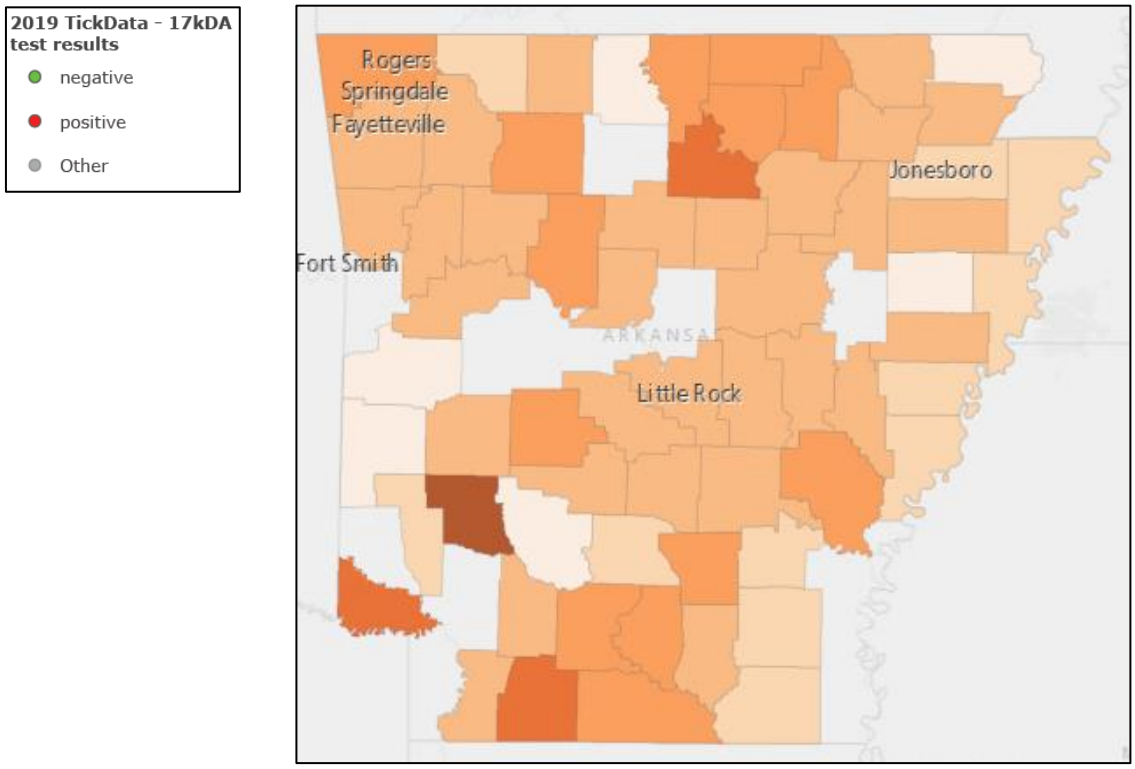

Figure 3. Map of Arkansas displaying prevalence of *Rickettsia* with a colored scale based on levels of significance.

Table 2.

Species of Ticks with Associated Common Names and Prevalence

Genus 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 following calculation: $\frac{No.\ Ticks\ Screened}{4676}\ x\ 100$

Table 3.

Species of Ticks with Associated Common Names and Prevalence of SFGR

Genus 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 following calculation: $\frac{No.\ Ticks\ Positive\ for\ SFGR}{No.Ticks\ Screened}\ x\ 100$

Table 4.

Species of SFGR Found in Ticks Sampled

	Known Disease Causing SFGR			
	R. amblyommatis	*R. andeanae*	*R. rickettsii*	*R. raoultii*[a]
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

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, P., Paddock, C., Socolovschi, C., Labruna, M., Mediannikov, O., Kernif T., Abdad, M., Stenos, J., Bitam, I., Fournier, P., & Raoult, D., 2014, *American Society for Microbiology*.

[a] This species was only found in the presence of *R. montanensis*.

Discussion

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* (SFGR). This is the one of the only existing studies conducted utilizing geospatial analysis techniques to determine the geographic distribution for SFGR in Arkansas, and therefore the areas that pose the greatest threat to human and animal health in the state. This study used citizens to gain access to samples from across Arkansas. Samples were grouped based on which county in Arkansas they originated from. This is because health departments tend to divide disease risk based on county. Local health departments inform, educate, and investigate health risks facing their communities. For this reason, it was determined prevalence based by county would be the most useful determinate of health risk.

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, prevalence of SFGR was applied to a map in order 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 (Figure 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 0-1119 ticks. Unfortunately, with studies that depend on citizens, there is the risk that there will be lack of participation. This was true for 11 of Arkansas's 75 counties. Some counties reported remarkably high or low prevalence, but the sample size in which that data was obtained was very small. For instance, Pike county is shown to have 100% prevalence, but only one tick was supplied for the entire county. While the tick was positive for *R. andeanae*, this does not reflect Pike's county actual prevalence of SFGR. A large portion of samples were obtained from Northwest Arkansas, specifically Washington and neighboring counties. These counties better reflect their actual exposure risk to SFGR due to the increased sample size. Pike, Little River, Stone, Columbia, Izard, Fulton, Arkansas, Ouachita, Sharp, Newton, Garland, and Union county reflected prevalence of greater than 50%. These counties should be considered high exposure risk for SFGR. It is important to note that sample size was inconsistent throughout this group. Areas found with prevalence of less than 25% should be considered low-moderate low rick for SFGR exposure. The following counties are included in this range: Phillips, Howard, Drew, Ashley, Dallas, Lincoln, Mississippi, Crittenden, Craighead, Lee, Clark, Clay, Cross, Marion, Polk, and Scott. Once again, sample size must be considered with this data as some of the counties displaying exceptionally low prevalence had very few samples to analyze. The remaining 36 counties should be deemed moderate to moderate high-risk exposure areas.

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 were found to contain *Rickettsia*. This is largely concerning since *A. americanum* is considered to be an aggressive human-biting tick and has vector competence capabilities (Levin et al., 2017). Each of the six tick species found throughout this study are considered capable of transmitting rickettsial species (Lee et al., 2018; Levin et al., 2017). *Ixodes scapularis* was found to have the highest 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 were 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 *R. montanensis*, *R. massiliae*, *R. rhipicephali*, and *R. amblyommatis* (Li et al., 2017). Ticks testing positive for *R. montanensis* were not always found with *R. raoultii*. Of the rickettsial pathogens found in samples, *R. montanensis* and *R. bellii* are consider of less significant as their capability to transmit disease has yet to be proven (Mullen & Durden, 2009), but research has begun to suggest that *R. bellii* could eventually be found to be disease causing (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 & Durden, 2009). This is concerning information since the most common pathogen, *R. amblyommatis*, was found in the most common tick, *A. americanum.* Similar findings regarding the common association of *R. amblyommatis* with *A. americanum* has been found in numerous studies (Moncayo et al., 2010; Stromdahl et al., 2008).

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. The lack of data in those areas leaves the study with an incomplete picture of the Arkansas tick population, but more importantly an incomplete distribution of SFGR. Regardless of sample size, the disease risk is evident throughout Arkansas. 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 implication that rickettsial species have on their host. Some species of the SFG *Rickettsia* are known to have lethal effects on their tick host (Niebylski, Peakcock, & Schwan et al., 1999). Furthermore, time of year the specimen is obtained could be relevant information regarding the when humans and animal are at most risk for being exposed to ticks.

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