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Prevalence of Ehrlichia and Rickettsia Within Ticks in Arkansas State Parks

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Table of Contents

Abstract

Within the past few decades, the number of diagnoses of tick-borne diseases – such as spotted fever rickettsiosis and ehrlichiosis – has steadily increased throughout the United States, with Arkansas having one of the highest rates in the country. These diseases pose a risk to both humans and animals, as both can acquire these infections from ticks. Due to the increasing geographic range of ticks, rise in cases, and likely underdiagnosis of these diseases, this study aimed to identify the infection rate of ticks themselves in multiple state parks in Arkansas. Four Arkansas state parks were each visited once a month for four months, and ticks were collected through a combination of dragging and dry ice methods. A total of 1,255 ticks were collected, of which 1,021 were nymphs and 234 were adults. Twelve ticks were identified as *Ixodes scapularis*, and the other 1,243 were identified as *Amblyomma americanum*. Depending on the park, the total *A. americanum* adults infected ranged from 55% to 72% for *Rickettsia*, 2.65% to 25% for *Ehrlichia ewingii*, and 0% to 8.33% for *Ehrlichia chaffeensis*. The total *A. americanum* nymphs infected ranged from 20.11% to 21.67% for *Rickettsia*, 0.27% to 1.78% for *E. ewingii* and from 0% to 1.67% for *E. chaffeensis.* The proportion of ticks infected with these pathogens did not significantly vary from park to park, but there was a significant association between the parks and sample size for dragging, indicating tick density varied significantly from park to park. This study demonstrates the high risk of tick-borne disease within Arkansas and that tick density varies substantially ($P < 0.02$) within the state.

Introduction

Background and Need

In the United States, pathogens carried by ticks are the cause of approximately 95% of all reported vector-borne diseases (Sonenshine, 2018). The number of reported diagnoses of tick-borne diseases in humans has steadily been on the rise within the past few decades, and these cases represent a fraction of the number of people who have actually acquired a tick-borne disease (Centers for Disease Control and Prevention [CDC], 2019a). Tick-borne diseases include five main groups: Rocky Mountain spotted fever (RMSF), other spotted fever group (SFG) rickettsioses, ehrlichiosis caused by *Ehrlichia chaffeensis*, other ehrlichioses, and anaplasmosis (Biggs et al., 2016). Additionally, the geographic ranges of ticks that carry disease-causing pathogens have increased over time significantly, and the tick species present vary from area to area (Sonenshine, 2018). Specifically, 63% of reported cases of rickettsioses, including RMSF, only come from five states – Missouri, Oklahoma, Tennessee, North Carolina, and Arkansas (Biggs et al., 2016). Four of these states – Missouri, Oklahoma, Tennessee, and Arkansas – also have the highest rates of ehrlichiosis (Biggs et al., 2016). Arkansas was chosen as the focus of this project due to these high disease rates and feasibility for the researcher.

Rocky Mountain spotted fever, caused by *Rickettsia rickettsii*, has a fatality rate estimated at 5%-10% in humans, with fatality rates increasing to 40%-50% when left untreated until the 8th or 9th day (Biggs et al., 2016). *Ehrlichia chaffeensis* ehrlichiosis has a lower fatality rate in humans estimated at 3%, and there have been no deaths ever reported in humans due to *E. ewingii* ehrlichiosis (Biggs et al., 2016). All three pathogens can also infect companion animals, with dogs being the preferred host for the brown dog tick species; diagnosis of RMSF in pet dogs

has even elicited recognition of RMSF in humans due to similar illness (Biggs et al., 2016). Therefore, researching the prevalence of disease-causing pathogens in ticks is not only pertinent to doctors and the health of humans, but it is pertinent to veterinarians and the health of pets as well.

Problem Statement

As stated by the CDC (2019a), the number of reported cases in humans only represents a fraction of those that have acquired a tick-borne disease, and the number of reported cases is on the rise. Thus, the number of reported cases of tick-borne diseases in humans is not an accurate number to gauge the prevalence of disease-causing bacteria in a tick population, and due to the rise in cases, it is important to identify how many and which ticks currently carry these bacteria. Due to the increasing range expansion of ticks such as the Gulf Coast tick (Sonenshine, 2018), it may also be useful to analyze how tick populations vary within different locations of a state itself. For this study, ticks were collected from various Arkansas state parks due to their popularity for hiking. A study demonstrating the prevalence of *Rickettsia* and *Ehrlichia* in various Arkansas state parks could help determine a park's risk level, what precautions to take, and if a human or animal does get a tick bite, what the likelihood is that it contained a disease-causing pathogen.

Purpose Statement

The purpose of this study was twofold: (1) To identify the different tick species in Arkansas state parks and to analyze how the proportion of ticks with *Ehrlichia* and *Rickettsia* varied from park to park; and (2) to estimate the relative likelihood of a person encountering a tick carrying *Ehrlichia* or *Rickettsia* pathogens.

Research Objectives

To meet the purpose, three objectives were developed:

1. Quantify the number of ticks collected for each Arkansas state park.
2. Describe the proportion of ticks that carried *Ehrlichia* and *Rickettsia* for each Arkansas state park and how the proportion differed for each park.
3. Describe the different tick species in Arkansas state parks and how the proportion of ticks that carried *Ehrlichia* and *Rickettsia* differed for each tick species.

Literature Review

Due to the increasing rate of reported tick-borne diseases in the United States and the danger of tick-borne diseases to animals and humans, studying the prevalence of tick-borne pathogens is paramount. Two of these disease groups – spotted fever rickettsioses (SFR) and ehrlichiosis – were the focus of this study. In humans, symptoms of RMSF – a disease included in SFR – include vomiting, fever, chills, headache, rash near the site of the tick bite, and muscle ache, with a fatality rate of 5-10% overall (Biggs et al., 2016). However, a wide variety of symptom combinations exist; the classic triad of an observed tick bite, rash, and fever is only present in a minority of patients, and a lack of diagnosis can increase the fatality rate to 40-50% (Biggs et al., 2016). Additionally, in dogs, RMSF causes anorexia, fever, skin rash, and ocular petechiae, and those with adult ticks have more severe symptoms (Levin et al., 2014). Ehrlichiosis has similar symptoms as RMSF in humans, including nausea, fever, headache, muscle ache, and irregular rash, but has a lower fatality rate of 3% overall; also similar to RMSF, ehrlichiosis is present in domestic animals (Biggs et al., 2016). Thus, these diseases can be very

dangerous for humans and animals, especially without proper diagnosis and awareness of tick-borne illnesses.

The danger of these diseases in the United States is amplified by the recent rise in reported cases. Within the past two decades, the number of reported cases of ehrlichiosis has multiplied by approximately 9 times, and the number of reported cases of SFR has multiplied by approximately 12.6 times (CDC, 2020a, 2020b). Concerningly, reported cases represent a fraction of the actual number of cases due to underdiagnosis, so the number of annual tick-borne illnesses is unknown (CDC, 2019a). However, pathogens that cause these tick-borne diseases can be detected in ticks themselves, and the species and incidence of these pathogens differ between tick species and geographic regions (Fryxell et al., 2015; Sonenshine, 2018). Consequently, knowing the infection rate in various tick species and geographic locations may provide a more accurate measurement of the risk level of a tick-borne disease than the number of reported cases. With this assumption and the objectives of the study in mind, the goal of this literature review is to analyze the previous research regarding geographic differences for tick-borne pathogens, compare tick collection methods, and discuss molecular tests for pathogens.

Geographic Range of Ticks

As the rate of reported cases of tick-borne diseases has risen, so has the general geographic range of the ticks that carry these diseases. Specifically, ticks have been expanding northward and westward, and ticks have become increasingly more adaptable, able to form populations with only small initial numbers (Sonenshine, 2018). This has led to the growth of ticks in extremely different biomes and future uncertainty about tick expansion due to human activity (Sonenshine, 2018). Specifically, reforestation, warmer temperatures, and the increase of

white-tailed deer populations all contribute to changes in tick populations (Eisen et al., 2017). These findings indicate that studies of tick geographic ranges need to be continually conducted to account for adaptability of tick populations and changes in the environment.

In Arkansas, the focus of this study, there has been some research on the prevalence of disease but no recent research on the differences between multiple locations such as state parks. In one recent study, 29% of ticks collected from dogs and white-tailed deer in Arkansas were positive for *Rickettsia* genes, and some tick species had significantly higher rates of pathogens than others (Fryxell et al., 2015). However, since the ticks were collected from animals, no specific geographic areas could be determined since the animals' geographic history and ranges were unknown. In a study conducted in Little Rock, Arkansas's largest city, researchers found pathogens linked to spotted fever rickettsioses (SFR) and ehrlichiosis in ticks (Blanton et al., 2014). This suggests that disease-carrying ticks may also exist in areas with higher human activity, such as popular hiking trails in Arkansas state parks.

Tick Collection Methods

To determine rates of tick-borne diseases in Arkansas, ticks must be carefully collected. Tick collection comes in a wide variety of methods, including collecting ticks from animal hosts, community science, or collecting ticks directly from the environment. In a review of previous research utilizing the host collection method, Lydecker et al. (2019) concluded that this method is not standardized across studies, the type of host can affect tick loads, and the process is deceptively complex. Additionally, one can logically conclude that because animals can travel long ranges, determining specific geographic areas from ticks on animal hosts is impossible. The community science tick collection method involves community members reporting tick

encounters and/or sending in ticks for pathogen testing (Eisen & Eisen, 2020). While useful in many aspects, community science comes with limitations of possible inaccurate location reporting and heavy skewing towards ticks in the adult life stage (Eisen & Eisen, 2020). The last method – collecting ticks directly from the environment – allows for greater control and monitoring by the experimenter.

Collecting ticks directly from the environment has several different sub-methods, including dragging, flagging, dry ice, sweep netting, CO_2 dragging, and CO_2 flagging. In a study between these methods, dry ice was discovered to be the most consistent across different habitats and time, whereas dragging was the second most consistent and the most cost-effective (Mays et al., 2016). While CO_2 methods were also comparable to dry ice and dragging in most instances, the weight of the CO_2 tank and cost of the equipment created drawbacks that outweighed some of the benefits (Mays et al., 2016). In the trials, sweep netting was deemed the least effective, and flagging was deemed less effective than most other methods (Mays et al., 2016). While dry ice and dragging methods were established as generally the most effective tick collection methods, another study found that these two methods varied significantly regarding tick life stage and species, so a single sampling method may not be adequate to get a representative sample of a tick population (Petry et al., 2010). In particular, dry ice is typically less effective for ambush tick species, in which the tick waits for a host to walk by rather than hunt a host (Petry et al., 2010). Thus, it can be concluded that a combination of dragging and dry ice is ideal to provide a comprehensive method of tick collection.

Molecular Testing of Pathogens

Once tick collection has finished, ticks must be tested for pathogens via molecular testing in a laboratory. Polymerase chain reaction (PCR) methods are modern technologies that allow researchers to avoid bacteria cultures and allow for specific and sensitive testing of diseases (Raoult & Roux, 1997). Deoxyribonucleic acid extraction, amplification, and PCR assays remain effective methods for testing ticks for disease-causing pathogens (Biggs et al., 2016). For *Rickettsia* species, the genes targeted through PCR assays include the 16S rRNA gene, citrate synthase gene, 17-kDa protein, and the rOmpB and rOmpA outer membrane proteins (Blanton et al., 2014; Fryxell et al., 2015; Raoult & Roux, 1997). Similarly, ehrlichiosis can be detected with the amplification of the 478-bp region and 379-bp fragment of the 16S rRNA gene (Pat-Nah et al., 2015). *Rickettsia*, *Ehrlichia ewingii*, and *Ehrlichia chaffeensis* can be simultaneously detected via a multiplex qPCR using hybridization probes and primer sets (Dowling et al., 2021).

Methodology

Considering previous research and the purpose of this study, collecting ticks directly from the environment was the best method available to ensure accurate reporting of tick geographic locations. Specifically, a combination of dragging and dry ice was used in this study in attempt to increase consistency between tick species, habitats, and life stages, which improved the likelihood of acquiring a representative sample of the tick population for each park. However, due to the constraints of the study, only adult and nymphal ticks were collected; the sample referenced as “ticks” throughout this study excludes the larval life stage. The molecular testing in this study was also based on previous research regarding PCR methods, DNA extraction, and gene amplification. These methods were the result of a nonexperimental,

quantitative research design, which aligned with the purpose of the study. Additionally, to make sure the results of this study were accurate and reliable, certain categories of rigor – including confounding variables, selection bias, population validity, and instrumentation – were accounted for through the study's methods.

Research Design

This study utilized a nonexperimental, quantitative descriptive research design. The general aim of quantitative research is to establish relationships, explain causal relationships, or describe a situation, and one of the main goals of the researcher is to be as objective as possible (Mertler, 2015, p. 108). Unlike experimental quantitative research, descriptive quantitative research does not manipulate any variables, and variables are observed and measured as they naturally occur (Mertler, 2015, p. 111). The aim of descriptive research, then, is to describe and interpret a current situation or status by measuring frequency, intensity, proficiency, or accuracy using scales and instruments (Mertler, 2015, p. 111). This was an appropriate research design for this study because it aligned with the purpose – to objectively measure the natural frequency of *Ehrlichia* and *Rickettsia* species in ticks in Arkansas state parks. Using this design allowed for the count of the number of ticks acquired, along with the description of the frequency of *Ehrlichia* and *Rickettsia* in each tick species and park.

Rigor

Confounding Variables

Confounding variables are variables that are not intentionally manipulated in the study by the researcher but may influence the results (Onwuegbuzie, 2000). It is the goal of the researcher to control and reduce these as much as possible (Onwuegbuzie, 2000). In tick

collection, when to sample matters substantially for accurate results, and sampling should only be conducted during peak adult tick activity since tick activity changes throughout the year (CDC, 2019b). Sampling should also not be conducted when it is raining or when there is wet vegetation, as it could saturate the drag (CDC, 2019b). To account for these confounding variables, tick collection was only conducted during the spring and summer, which is the active tick season in Arkansas, and ticks were never collected when there was wet vegetation to avoid skewing of results. Ticks were also not collected during early morning or late at night to prevent dew from collecting on the drag.

Selection Bias

To avoid selection bias, multiple methods of tick collection were used. Selection bias refers to significant differences between two or more groups, which becomes apparent at the data collection stage (Onwuegbuzie, 2000). The greater the selection bias, the more risk there is to internal validity (Onwuegbuzie, 2000). Since different tick collection methods target different tick species, one must use multiple methods of tick collection to get a representative sample in an area (Mays et al., 2016; Petry et al., 2010). Thus, to avoid inadvertently selecting only certain tick species, this study used a combination of dragging and dry ice tick collection methods. These methods were chosen to acquire a representative sample of the tick population in each Arkansas state park.

Population Validity

To have population validity, the findings must be generalizable from a sample of individuals to the larger population (Onwuegbuzie, 2000). Generally, the larger and more random the samples, the more the population validity increases (Onwuegbuzie, 2000). While the

collection of ticks at each Arkansas state park could not be truly randomized in this study, strict collection procedures were conducted based on previous research to obtain as close to a representative sample as realistically possible. Additionally, tick collection was conducted over a large time period, which is standard for the field, to allow for a larger sample size and sample diversity.

Population and Sampling

The population in this study consisted of adult and nymphal ticks in Arkansas state parks. Due to varying effectiveness of tick collection methods regarding different tick species and habitats (Mays et al., 2016; Petry et al., 2010), ticks were collected using a combination of dragging and dry ice in an attempt to acquire a representative sample of tick species in each Arkansas state park. It's important to note that these tick collection methods use convenience samples since the ticks collected are determined by proximity and cannot be randomized. However, strict sampling procedures were undertaken following the procedures of previous tick collection studies to reduce the chance of a non-representative sample as much as possible.

Dragging was conducted at each park in 800 strides by the researcher, which is one of the many methods used to determine distance sampled in tick collection (CDC, 2019b). This method was chosen due to the time and resource constraints of the researcher. The "drag" consisted of a 1-m^2 cloth that was pulled completely flat against the habitat floor, as proven effective in previous studies (Mays et al., 2016; Ogden et al., 2014). Additionally, following the standard for the dry ice method, coolers of dry ice were left out for 24 hours at each state park to maximize the amount of time for ticks to find and reach the carbon dioxide source (Kensinger & Allan, 2011; Mays et al., 2016). This prevented bias towards faster ticks and improved sample

size. With these standardized sampling procedures, the ticks collected were quantified and the tick species at each park were described.

Data Collection

Ticks were sampled from May to August from various Arkansas state parks. Due to the financial and temporal constraints of the study, only 4 state parks were visited. However, all 4 were chosen to represent popular parks, and each was visited once a month for a total of 16 samples. These parks were Pinnacle Mountain State Park, Moro Bay State Park, Petit Jean State Park, and Lake Catherine State Park. Pinnacle Mountain State Park, Petit Jean State Park, and Lake Catherine State Park are all located in central Arkansas, whereas Moro Bay State Park is located near the southern border. At each park, ticks were collected with a combination of dragging and dry ice methods. In the woods of each park, dry ice was placed in a cooler with two drill holes, which allowed carbon dioxide to escape. Under the cooler was a piece of cardboard surrounded by double-sided carpet tape, which adhered to the ticks, as proven effective in previous studies (Kensinger & Allan, 2011). These coolers were left out for 24 hours at each location before collection to allow time for ticks to arrive at the cooler (Kensinger & Allan, 2011; Mays et al., 2016).

After the coolers were set up, dragging was conducted for 800 strides. The researcher's strides were measured as approximately 60 cm, which means in total, dragging was conducted for approximately 480 meters at each park visit. The drag consisted of a rod attached to a 1-m^2 white flannel cloth so that it could lie completely flat on the ground behind the researcher, as is the standard (Mays et al., 2016; Ogden et al., 2014). The drag was periodically checked every 20 strides, or approximately every 12 meters, to remain within the standard 10–15-meter range and

collect ticks before they had a chance to drop back off and possibly escape (CDC, 2019b). Each tick from the drag cloth was stored in a vial with 95% ethanol for preservation, and each vial was labeled with the date and name of the state park. Similarly, after 24 hours for the dry ice method, each tick on the tape, cardboard, and cooler were preserved through the same process. Once all ticks were collected, they were stored in a freezer until they could be tested later that fall.

In the fall, tick species were identified via microscopy following tick identification guides. The residual ethanol was dried from the tick on a kimwipe before DNA extraction. Deoxyribonucleic acid was extracted from ticks following the manufacturer guide for Invitrogen PureLink Genomic DNA Mini Kits with the exception of ticks being incubated at 55°C overnight rather than 1-4 hours; this is the standard practice for our laboratory (Dowling et al., 2021). A multiplex qPCR was then conducted on the DNA extractions to simultaneously determine the presence of *Rickettsia*, *Ehrlichia ewingii*, and *Ehrlichia chaffeensis*, as used in a recent study (Dowling et al., 2021). This was prepared with 5.5 µl of the PrimeTime Gene Expression Master Mix, 2.1 µl of distilled water, 0.2 µl of each primer and probe (for a total of 1.4 µl per reaction), and 2 µl of DNA; the total final reaction was 11 µl. Each plate had a positive and negative control. The positive control consisted of a sample from the laboratory and was strongly positive for *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, and *Rickettsia*; the negative control contained everything except the DNA. For *Rickettsia* species, 17-kDa fragments were targeted using *Rickettsia* specific primers (Blanton et al., 2014; Fryxell et al., 2015; Raoult & Roux, 1997). *Ehrlichia* species were targeted by using *Ehrlichia* specific primers on the 16S rRNA gene (Pat-Nah et al., 2015).

Data Analysis

The methods of this study were carefully conducted – following standards set in previous research and considering elements of rigor – to reduce bias as much as possible. These methods were in the form of a nonexperimental, quantitative research design, which allowed for the measurement of the natural frequency of *Ehrlichia* and *Rickettsia* species within ticks in the environment. Although the ticks collected in this study were convenience samples, dragging and dry ice were used in combination, following past research, to prevent potential biases and to acquire as close to a representative sample as possible for the tick population in each park. After ticks were collected, real-time qPCR technology was then used to test the ticks' bodies for pathogens, which also followed the standard in past research. Once molecular testing of the ticks' genes for *Ehrlichia* and *Rickettsia* was completed, the data collected throughout the sampling and testing procedures were collated and analyzed for significance using R Studio. Tests consisted of proportion tests and chi-squared tests to determine 1) if there was a significant association between the proportion of ticks infected with *Ehrlichia* and *Rickettsia* and parks and 2) if there was a significant association between parks and tick sample sizes from dragging.

Results

In total, 1,255 ticks were collected, and two tick species were identified. Of these, 1,021 were nymphs and 234 were adult males and females. Only 12 ticks collected were identified as *Ixodes scapularis*, and the other 1,243 were identified as *Amblyomma americanum*. The total number of *A. americanum* collected from each park is identified in Table 1, and the total number of *I. scapularis* collected from each park is identified in Table 2. Nymphs are indicated by the letter N and adults are indicated by the letter A.

Due to the high sample size of ticks in the nymph life stage, they were pooled into samples of approximately 4-5 nymphs. This entire pool was tested via qPCR to identify whether it was positive for one of the bacteria. If the pool was positive, only one nymph was assumed to be positive per pool; this was called the minimum infection prevalence (MIP). The *A. americanum* ticks were further broken down into dragging and dry ice groups to analyze potential differences between the sampling methods. The *A. americanum* dragging results may be observed in Table 3 and the dry ice results may be observed in Table 4.

Table 1 – *Ixodes scapularis* ticks collected from Arkansas state parks screened via qPCR for *Ehrlichia ewingii*, *Ehrlichia chaffeensis*, and *Rickettsia*.

Ixodes scapularis Total						
Field site	Life Stage	No. ticks	No. pools	No. infected (% infected)		
				E. ewingii	*E. chaffeensis*	*Rickettsia*
Pinnacle	N	5	1	0 (0.00%)	0 (0.00%)	1 (20.00%)
	A	1		0 (0.00%)	0 (0.00%)	1 (100.00%)
Petit Jean	N	1	1	0 (0.00%)	0 (0.00%)	0 (0.00%)
	A	0		0 (0.00%)	0 (0.00%)	0 (0.00%)
Moro Bay	N	1	1	0 (0.00%)	0 (0.00%)	1 (100.00%)
	A	1		0 (0.00%)	0 (0.00%)	1 (100.00%)
Lake Catherine	N	3	3	0 (0.00%)	0 (0.00%)	2 (66.67%)
	A	0		0 (0.00%)	0 (0.00%)	0 (0.00%)

Table 2 – *Amblyomma americanum* ticks collected from Arkansas state parks screened via qPCR for *Ehrlichia ewingii*, *Ehrlichia chaffeensis*, and *Rickettsia*.

Amblyomma americanum Total						
Field site	Life Stage	No. ticks	No. pools	No. infected (% infected)		
				E. ewingii	*E. chaffeensis*	*Rickettsia*
Pinnacle	N	169	39	3 (1.78%)	2 (1.18%)	36 (21.30%)
	A	113		3 (2.65%)	1 (0.88%)	63 (55.75%)
Petit Jean	N	373	80	1 (0.27%)	0 (0.00%)	75 (20.11%)
	A	57		5 (8.77%)	0 (0.00%)	29 (50.88%)
Moro Bay	N	60	14	1 (1.67%)	1 (1.67%)	13 (21.67%)
	A	12		3 (25.00%)	1 (8.33%)	8 (66.67%)
Lake Catherine	N	409	86	3 (0.73%)	2 (0.49%)	86 (21.03%)
	A	50		5 (10.00%)	1 (2.00%)	36 (72.00%)

Table 3 – *Amblyomma americanum* ticks collected from dragging in Arkansas state parks screened via qPCR for *Ehrlichia ewingii*, *Ehrlichia chaffeensis*, and *Rickettsia*.

Amblyomma americanum Dragging Only						
Field site	Life Stage	No. ticks	No. pools	No. infected (% infected)		
				E. ewingii	*E. chaffeensis*	*Rickettsia*
Pinnacle	N	154	33	3 (1.95%)	2 (1.30%)	33 (21.43%)
	A	29		0 (0.00%)	0 (0.00%)	23 (79.31%)
Petit Jean	N	360	77	1 (0.28%)	0 (0.00%)	73 (20.28%)
	A	23		3 (13.04%)	0 (0.00%)	16 (69.57%)
Moro Bay	N	46	11	1 (2.18%)	1 (2.18%)	10 (21.74%)
	A	9		3 (33.33%)	1 (11.11%)	6 (66.67%)
Lake Catherine	N	354	73	1 (0.28%)	1 (0.28%)	74 (20.90%)
	A	17		2 (11.76%)	1 (5.88%)	11 (64.71%)

Table 4 – *Amblyomma americanum* ticks collected from dry ice in Arkansas state parks screened via qPCR for *Ehrlichia. ewingii*, *Ehrlichia chaffeensis*, and *Rickettsia*.

Amblyomma americanum Dry Ice Only						
Field site	Life Stage	No. ticks	No. pools	No. infected (% infected)		
				E. ewingii	*E. chaffeensis*	*Rickettsia*
Pinnacle	N	15	6	0 (0.00%)	0 (0.00%)	3 (20.00%)
	A	84		3 (3.57%)	1 (1.19%)	40 (47.62%)
Petit Jean	N	13	3	0 (0.00%)	0 (0.00%)	2 (15.38%)
	A	34		2 (5.88%)	0 (0.00%)	13 (38.24%)
Moro Bay	N	14	3	0 (0.00%)	0 (0.00%)	3 (21.43%)
	A	3		0 (0.00%)	0 (0.00%)	2 (66.67%)
Lake Catherine	N	55	13	2 (3.63%)	1 (1.81%)	12 (21.81%)
	A	33		3 (9.09%)	0 (0.00%)	25 (75.76%)

There was no statistically significant difference between the prevalence of infection for *Amblyomma americanum* among the different state parks, including the total, dragging, and dry ice breakdowns ($P > 0.05$). However, due to the small sample sizes of certain infections, the chi-squared test used to determine significance was possibly inaccurate and the proportion tests were somewhat underpowered. It is also important to note that due to dry ice unavailability for two site visits (Petit Jean in May and Moro Bay in June), the dry ice results were more unreliable than dragging. Assuming there would be equal distribution among the parks regarding tick

density and thus sample size of ticks collected during dragging, there was a significant association between parks and the number of *A. americanum* ticks collected for both adult ($P < 0.02$) and nymph ($P < 0.01$) life stages. Moro Bay had 55 ticks total, Pinnacle Mountain had 183 ticks total, Lake Catherine had 371 ticks total, and Petit Jean had 383 ticks total. *Ixodes scapularis* ticks were not analyzed due to the small sample size, but they represented 0.85% of total adult ticks and 0.98% of total nymph ticks collected.

Discussion

This study showed that although the proportion of ticks positive for these infections did not significantly vary among parks, Arkansas state parks have a high percentage of ticks that can transmit disease-causing bacteria. The percentage of total adult *A. americanum* infected ranged from 50.88% to 72.00% for *Rickettsia*, 2.65% to 25% for *Ehrlichia ewingii*, and 0% to 8.33% for *Ehrlichia chaffeensis*. The percentage of *A. americanum* adults (n = 232) infected with *Rickettsia* across all the parks was 58.62%. For *Rickettsia*, this was higher than two other studies conducted in Arkansas regarding the infection rate in *A. americanum* (Dowling et al., 2021; Fryxell et al., 2015). With a sample size of 4,006 adult and pooled nymphs of *A. americanum*, a previous study found a *Rickettsia* infection rate of 47.6% (Dowling et al., 2021). The study pooled nymphs into groups of up to five and included positive pools in the total (Dowling et al., 2021). Thus, with nearly every nymph pool being positive in this study, the percentage of total ticks infected with *Rickettsia* would be much higher than adults only in this study with a similar analysis. Another study had an infection rate of 32% for 237 adult *A. americanum* males and 41% for 233 adult *A. americanum* females (Fryxell et al., 2015). The much higher infection rate in this study is of note. The *Ehrlichia* infection rate of *A. americanum* in this study was similar to the 6.6% infection rate of the 4,006 adults and pooled nymphs in the study by Dowling et al. (2021).

Further research could attempt to determine if this was an abnormal collection period or if the varying collection seasons in the studies account for this disparity. Additionally, as the studies, including this one, became more recent, the *Rickettsia* infection rate in ticks consistently increased, which could be investigated. Since one previous study was conducted via community science (Dowling et al., 2021), the other via host collection (Fryxel et al., 2015), and this study via dragging and dry ice, future research could also explore whether the type of methodology used impacted the results. As for the nymph pools, the minimum infection prevalence of *Rickettsia* was consistently around 20%. This closely matched with the pools being 4-5 ticks; nearly every pool was positive. Additionally, the percentage of total nymph ticks infected ranged from 0.27% to 1.78% for *E. ewingii* and from 0% to 1.67% for *E. chaffeensis.* Future research could reduce the nymph pool size to determine a more accurate infection rate.

Further analysis of these results also answered the question of relative likelihood of encountering a tick infected with one of these bacteria. As proportions of ticks infected did not statistically vary from park to park, the tick density of dragging alone can be observed to analyze this. By far, Moro Bay had the smallest tick density at 55 ticks over the course of the 4 collection periods in the summer of 2021. Pinnacle Mountain had the next smallest with a total of 183 ticks. Lake Catherine and Petit Jean were relatively similar with 371 and 383 ticks, respectively. Thus, Moro Bay would be the least likely park to encounter an infected tick, Pinnacle Mountain would be the next likely, and then Lake Catherine/Petit Jean would be the likeliest. All parks had far greater nymph populations than adult populations. Further research should explore why there are such disparities between the tick populations between the parks, especially Moro Bay, with only 14% of the tick density compared to the highest-density park. It is important to consider there is

room for human error with possible varying stride lengths among the parks, but it is unlikely to cause such a vast difference.

Ultimately, this research could be used as a foundation for further studies concerning tick-borne diseases in Arkansas. This study also demonstrates the high public health risk to both humans and animals regarding infected ticks while visiting Arkansas state parks, supporting the findings that Arkansas has one of the highest spotted fever and ehrlichiosis diagnoses rates in the country (Biggs et al., 2016).

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