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Survey of Ticks and Tick-Borne Pathogens Associated with Feral Swine (*sus scrofa*) in Arkansas

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Survey of Ticks and Tick-Borne Pathogens Associated with Feral Swine (*sus scrofa*) in Arkansas

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Entomology

by

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Arkansas State University
Bachelor of Science in Wildlife Ecology and Management, 2018

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This thesis is approved for recommendation to the Graduate Council.

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Abstract

Feral hogs (*Sus scrofa* L.) are an invasive species throughout the southeast United States and found in every Arkansas county. As feral hogs invade new habitat, they can disrupt ecosystems, damage agriculture systems, and bring ticks and tick-borne pathogens with them. There are no surveys of the tick species parasitizing the Arkansas feral hog populations or the pathogens they carry. This is a public health concern because feral hogs occupy often same geographical regions as humans and livestock and can harbor over 45 animal diseases and parasites. The ticks carried by feral hogs can carry tick-borne pathogens that can cause diseases such as Ehrlichiosis, Rocky Mountain spotted fever and Lyme disease.

In this study, tick collections were taken from feral hogs trapped by Arkansas Game and Fish Commission, USDA APHIS Wildlife Services and University of Arkansas Cooperative Extension Service personnel for control purposes on private properties. From February 2019 to January 2020, 220 feral hogs were sampled for ticks in 11 Arkansas counties. Of the 3,009 ticks that were collected, 95.4% of them were *Amblyomma americanum* (L.), 2.4% were *Dermacentor variabilis* (Say), 0.6% were *Amblyomma maculatum* (Koch), and 0.3% were *Ixodes scapularis* (L.). Tick loads on the hogs ranged from 0-96. Most of the sampled hogs had single species infestations with 132 or 138 of these hogs being infested only *A. americanum* ticks. The remaining single species infestations were four hogs infested with *I. scapularis* and two with *D. variabilis*. Dual species infestations were seen on 42 hogs with 37 of these being infested with *A. americanum* and *D. variabilis*, four with *A. americanum* and *A. maculatum* and one with *A. americanum* and *I. scapularis*.

The ticks collected from the feral hogs were also used to test for pathogens. Pooled DNA extractions and PCR were used to survey the prevalence of *Rickettsia*, *Ehrlichia* and *Borrelia*

bacteria found in the ticks. Each pooled DNA extractions contained 1-10 ticks of the same host, species, life stage, and sex. From the DNA extractions used in this survey 75 were *A. americanum*, nine were *D. variabilis* and one was *A. maculatum*. PCR primers used targeted the citrate synthesis gene in *Rickettsia*, 16S gene in *Ehrlichia* and *flab* gene in *Borrelia*. Results from the PCR were 24 *Rickettsia* positives samples from 23 *A. americanum* pools and one *D. variabilis* pool. Five of the *Rickettsia* positives samples from *A. americanum* pools were purified, sequenced, and found to be a 100% match to *Rickettsia amblyommatis*. No *Borrelia* or *Ehrlichia* species were found in this survey. However, that does not mean that they are no present in the Arkansas feral hog populations as the hogs were found to host the tick species that carry those pathogens.

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Dedication

I would like to dedicate this work to two people. First, to the version of myself from five years ago who would not believe I was capable of this project and second, to Dr. Tina Grey Teague who saw something in me that I did not see in myself.

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Chapter 1: Introduction

Ticks (Order: Arachnida, Subclass: Acari) are obligate ectoparasites of mammals, birds, reptiles, and amphibians. There are three families of ticks (Ixodidae, Argasidae and Nuttalliellidae) with at least 850 species spread across all continents. Ticks are vectors and reservoirs of more viruses, bacteria, fungi, and protozoa than any other arthropod (Olson and Patz 2010). Ticks are the most important disease vector in veterinary settings and second only to mosquitos as human disease vectors (Fuente et al. 2008). Worldwide, 116 tick species are potential vectors of many medically important pathogens (Brites-Neto et al. 2015). As of 2019, there are 16 tick-borne pathogens in the United States transmitted by nine tick species (Table 1., Anonymous 2019^b).

In Arkansas, there are 15 Ixodid (hard ticks) and three Argasid (soft tick) species (Lancaster 1973). Five Arkansas Ixodid tick species, *Ixodes scapularis* (L.), *Amblyomma americanum* (L.), *Amblyomma maculatum* (Koch), *Riphecephalus sanguineus* (Latreille) and *Dermacentor variabilis* (Say), are associated with eight tick-borne human pathogens (Table 2) (Anonymous 2017^a). Since 2011, the numbers of reported tick-borne diseases have been increasing in Arkansas (Anonymous 2019^c). The tick – borne diseases that are reported most often are bacterial; rickettsiosis is the most prevalent with over 1,000 reported cases each year from 2017 - 2019 (Anonymous 2019^c). Rickettsiosis is followed by ehrlichiosis (235 cases), tularemia (71 cases), anaplasmosis (4 cases) and Lyme disease (4 cases) (Anonymous 2019^b).

Tick life history

Ixodid ticks go through a hemimetabolous life cycle with four distinct life stages: egg, larvae, nymph, and adult. All five of the medically important Ixodid species in Arkansas have a

three-host life cycle where each life stage must feed on a new host before molting (Anonymous 2019⁸).

After a gravid female drops from her host, she lays an egg mass that can range from 200-2,300 eggs (Klompen 2005). Before dying, females use two area porosae to secrete a wax layer over the eggs to help prevent desiccation (Klompen 2005). The eggs hatch and six-legged larvae, also known as “seed ticks,” emerge and begin questing for their first host. While questing, ticks perch on vegetation and extend their front tarsi which exposes the Haller’s organs to the air. The Haller’s organ consists of numerous chemo sensitive sensilla that can detect CO₂ and host heat trails (Klompen 2005). When a host gets close, the larvae climb on and feed for 3-7 days. Feeding rates of larvae, nymphs and adult females is differential; feeding begins slowly and progressively speed up with approximately half of the blood meal being ingested in the last 12-36 hours. After feeding from host 1, the larvae drop off, molt in the leaf litter, and emerge as eight-legged nymphs searching for host 2. After feeding for 3-8 days, the nymphs drop from host 2, molt in the leaf litter, and emerge as adults. Adult ticks can have two different mating strategies, 1) mating occurs on host 3 or 2) mating occurs in the leaf litter and only females search for host 3 (Klompen 2005). Once the third blood meal is complete, females drop from their hosts to lay eggs and the cycle continues.

Tick borne pathogens are typically acquired in the larval or nymph stage by feeding from an infected host and are then transmitted to new hosts during the nymph or adult stages (Parham et al. 2015). Pathogens are transmitted into the host’s blood during the feeding process by salivary gland secretions (Brites-Neto et al. 2015). The likelihood of pathogen transmission from a tick can be correlated with how long the tick was attached. For example, transmission of *Borrelia burgdorferi* (Johnson et al. 1984) from tick to host is positively correlated with the

amount of time the infected tick spent feeding (Parham et al. 2015). Coinfections of multiple pathogens in the same ticks have been reported with *B. burgdorferi*, *Babesia microti* (Franca, 1912), *Anaplasma phagocytophilum* (Foggie 1949) and *Rickettsia* spp. (Krause et al. 1996, Nadelman et al. 1997, Steiner et al. 2008).

Arkansas tick surveys

From 1990 to 1994, there was a North West Arkansas tick survey (Gullo 1998) that collected ticks from animal and human hosts, dry ice traps, and flagging vegetation. Of the 15,489 ticks collected, 7,560 were *A. americanum* (28.8%), 4,120 were *D. variabilis* (26.6%), and 3,809 were *I. scapularis* (24.6%). *Amblyomma americanum* would count for 54% of all collected ticks if only adults were considered.

From 1999 to 2000 there was a tick density study (Eads, 2001) conducted at Arkansas Post National Memorial, in southeast Arkansas by Gillett. Of the 44,832 ticks collected using cloth sweeps, 44,655 (99.6%) were *A. americanum* (Eads, 2001). The remaining ticks collected were: 92 *Haemaphysalis leporispalustris* (Packard), 41 *D. variabilis*, 30 *I. scapularis*, 13 *A. maculatum*, and one *Ixodes dentatus* (Marx) (Eads, 2001). *Amblyomma americanum* adult and nymph abundance were highest in oak/pine and sweetgum habitats (Eads, 2001). The high abundance is likely because these habitats have high percentages of leaf litter and canopy coverage and *A. americanum* ticks cannot survive in hot/dry environments (Eads, 2001).

More recently, in a 2010 study, ticks were collected from white tailed deer, canines, and felines in Arkansas (Trout et al. 2012). Five species were collected: 1636 *I. scapularis* (53.7%), 664 *A. americanum* (21.8%), 356 *R. sanguineus* (11.7%), 208 *A. maculatum* (6.8%), and 184 *D.*

variabilis (6%) (Trout et al. 2012). The high number of *I. scapularis* is due to the collections taken from white-tailed deer.

The Arkansas Tick-borne Disease Project is an on-going survey where Arkansas citizens send in ticks with the location they were collected. So far, over 10,000 ticks have been collected from humans, cats, dogs, horses, goats, raccoons, rabbits, potbelly pigs, cattle, deer, and feral hogs (Smith and Loftin 2018, personal comm.). From the 2017-2018 collection data: 76% were *A. americanum*, 16% *D. variabilis*, 3% *I. scapularis*, 3% *A. maculatum* and 2% *R. sanguineus* (Smith and Loftin, 2018, personal comm.). These data probably represent the tick species that are most encountered by humans and livestock rather than the actual percentages of species found in wild systems.

Haemaphysalis longicornis (Neumann), also known as the Asian Longhorn tick, is an emerging invasive species in the United States that has been found in North West Arkansas (Anonymous 2020). While no pathogen transmission cases have been documented in the United States, it has been demonstrated that these ticks can transmit *Rickettsia rickettsii* (Brumpt, 1922) under laboratory conditions (Stanley et al. 2020).

Rocky Mountain Spotted Fever

Since the 1920's Rocky Mountain spotted fever (*Rickettsia rickettsia*) has been a nationally notifiable disease but, starting in 2010, cases have been reported under the Spotted Fever Rickettsiosis category. The Spotted Fever Rickettsiosis category was created due to difficulty in distinguishing the different *Rickettsia* groups and contains *Rickettsia parkeri* rickettsiosis (Parker, 1939), *Rickettsia rickettsii*, *Rickettsia philipi* (type strain 3640) (Paddock, 2004) and *Rickettsia akari* (Greenberg, 1947) (Anonymous 2019^e). In the United States, Rocky Mountain

spotted fever (RMSF) is the most prevalent *Rickettsia* disease (Cohen et al. 2009). Arkansas, Oklahoma, Missouri, North Carolina, and Tennessee account for approximately 60% of Spotted Fever Rickettsiosis cases, although cases have been reported throughout the continental United States (Anonymous 2019^e). In 2018, there were 5,544 cases of Spotted Fever Rickettsiosis in the United States with 1,065 of those cases coming from Arkansas (Anonymous 2019^b, Anonymous 2019^e). In 2019, Spotted Fever Rickettsiosis was the most common tick-borne disease in Arkansas with 1,029 reported cases (Anonymous 2019^b).

It takes 4-6 hours of tick attachment to transmit the *Rickettsia* bacteria and usually seven days for an infected person to display the characteristic symptoms of fever, headache, and rash (Anonymous 2017^b). Although the number of cases of Spotted Fever Rickettsiosis has risen in the past decades, the number of fatal cases has been declining since the antibiotic tetracycline became available in the 1940's. Currently, fatality rates from Spotted Fever Rickettsiosis are 5-10% in the United States; in Brazil however, fatality rates range from 20-40% (Anonymous 2019^e) (Brites-Neto et al. 2015). Onset of Rickettsiosis cases are most common in June and July when the associated ticks are most active (Anonymous 2019^e).

The most important ticks in the United States regarding the transmission of Spotted Fever Rickettsiosis are *Dermacentor andersoni* (Stiles) and *Dermacentor variabilis* (Say) (Brites-Neto et al. 2015). In Arkansas, the main vector species is *D. variabilis* (Anonymous 2019^g).

Amblyomma maculatum infected with *R. parkeri* have been reported in multiple southern states; it is possible that *A. maculatum* is acting as a vector for Spotted Fever Rickettsiosis in Arkansas, as well (Cohen et al. 2009).

Ehrlichiosis

Ehrlichiosis is a bacterial disease that was recognized in the United States in 1986 when a patient from Fort Chaffee, Arkansas was infected with *Ehrlichia chaffeensis* (Anonymous 2017^a). It was not until 1999 that human monocytic ehrlichiosis (now called human ehrlichiosis) became a nationally notifiable disease (Anderson et al. 1991). Beginning in 2008, reported cases of ehrlichiosis were split into four categories determined by the bacteria causing the disease: *Ehrlichia ewingii* (Ewing 1971), *Ehrlichia chaffeensis* (Anderson 1991), *Anaplasma phagocytophilum* (Chen 1994) and undetermined *Ehrlichia/Anaplasma* bacteria (Anonymous 2019^d). *Anaplasma phagocytophilum* is often reported as anaplasmosis instead of ehrlichiosis, therefore, it will be discussed in more detail separately.

Ehrlichia ewingii was first described in 1992 when it was discovered as the bacteria that causes canine granulocytic ehrlichiosis in dogs (Anderson et al. 1992). *Ehrlichia ewingii* infects the neutrophil white blood cells of the host, when this occurs in humans it is called *E. ewingii* ehrlichiosis (Buller et al. 1999). *E. chaffeensis* infects the monocyte white blood cells of the host to cause human ehrlichiosis (formerly known as human monocytic ehrlichiosis) (Anderson et al. 1991, Chen et al. 1994).

Human ehrlichiosis infections are most often reported from the south central and south east United States with 35% of reported cases coming from Arkansas, Missouri, and Oklahoma (Anonymous 2017^a). Human ehrlichiosis is caused by both *E. chaffeensis* and *E. ewingii*, although, most cases in the United States are attributed to *E. chaffeensis* infections (Anonymous 2017^a). In 2018, there were 1,832 reported cases of human ehrlichiosis in the United States, of those cases 1,799 were caused by *E. chaffeensis* (98%) and 33 were caused by *E. ewingii* (2%) (Anonymous 2019^d). In 2019, Arkansas had 235 reported cases of human ehrlichiosis

(Anonymous 2019^d). All reported cases of human ehrlichiosis in Arkansas are reported under the category of Ehrlichiosis with no indication of which *Ehrlichia* species caused the disease. The most common symptoms of human ehrlichiosis are fever, chill, headache, muscle pain and joint pain with many patients also developing a rash (Dawson et al. 2005).

Most cases will occur in the spring and summer when the vector of human ehrlichiosis, *Amblyomma americanum* (L.), is most active and/or feeding on humans (Fishbein et al. 1994) (Anonymous 2017^a). After an infected bite, symptoms will take approximately 5-21 days to present themselves (Dumler et al. 2007). In the United States, *E. chaffeensis* is maintained by multiple wildlife species, although white-tailed deer (*Odocoileus virginianus*) (Zimmerman 1780) are the main reservoir (Paddock and Yabsley 2007). *Ehrlichia ewingii* DNA has also been detected in white tailed deer from a few southern states, including Arkansas (Yabsley et al. 2002). This is relevant because white-tailed deer are also the primary host for *A. americanum* in all life stages (Paddock and Yabsley 2007).

Tularemia

The first human illnesses associated with *Francisella tularensis* were documented in 1914 from two patients in Ohio who had contact with wild rabbits (Wherry and Lamb 1914). *Francisella tularensis* is an extremely infectious bacteria that causes disease, in many forms, in humans and animals (Ellis et al. 2002). The different forms of disease were thought to be separate until Edward Francis discovered that they were all caused by *F. tularensis* and gave them the name Tularemia (Francis 1921). Tularemia can present itself in glandular, ulceroglandular, oculoglandular, oropharyngeal, typhoidal and pneumonic forms, depending on the way the pathogen is introduced into the body. Oculoglandular, oropharyngeal, typhoidal and pneumonic forms are contracted from direct contact of *F. tularensis* through ingestion, skin, or

mucus membranes. Glandular and ulceroglandular forms of tularemia are contracted through the bite of an infected vector or contact with an infected animal (Petersen and Schriefer 2005).

Ulceroglandular tularemia causes enlarged lymph nodes and a lesion at the site of infection; glandular tularemia is very similar to ulceroglandular, except that it lacks a lesion (Petersen and Schriefer 2005). Locally acquired tularemia has been reported from every continental state, but over 50% of reported human cases are from Kansas, South Dakota, Oklahoma, Missouri, and Arkansas (Anonymous 2018^d).

In comparison to other tick – borne pathogens tularemia's numbers are relatively low with 229 cases in the United States in 2018 and 56 of those cases occurring in Arkansas (Anonymous 2018^d, Anonymous 2019^c). The tick vectors of *F. tularensis* in the United States are *D. variabilis*, *D. andersoni* and *A. americanum* (Anonymous 2019^c). *Francisella tularensis* is maintained in the environment through multiple wildlife species including rabbits, hares, beavers, muskrats, and voles (Morner 1992). When there are outbreaks in Arkansas, rodents, rabbits, and hares die in large numbers (Anonymous 2017^c).

Human granulocytic anaplasmosis

Human granulocytic anaplasmosis is a disease caused by the bacterium *A. phagocytophilum* which infects the neutrophil white blood cells of hosts (Thomas et al. 2009). *Anaplasma phagocytophilum* was first known in veterinary settings as *Ehrlichia phagocytophila*, the agent of tick-borne fever in ruminants, and *Ehrlichia equi*, the agent of equine granulocytic ehrlichiosis (Foggie 1951, Gribble 1969). After taxonomic reorganization in the early 2000's, the two previously separate bacteria are now grouped together as *Anaplasma phagocytophilum* (Dumler et al. 2001). Human granulocytic anaplasmosis was not recognized in the United States until 1994, when 12 patients were found to be infected with bacterium that resembled *E. chaffeensis*

and did not become a nationally notifiable disease until 1999 (Chen et al. 1994, Anonymous 2019^a).

Humans usually begin to show symptoms within 21 days after a bite from an *I. scapularis* tick infected with the bacteria (Dumler et al. 2007). The most common symptoms are high fever, headache, muscle pain, joint pain and a decrease in both white blood cells and blood platelet counts (Loebermann et al. 2006, Wormser et al. 2006). Although 36% of infected patients are hospitalized, the fatality rate is low and usually are a result of secondary infections that occur while the immune system is compromised (Bakken and Dumler 2015, Woldehiwet 2010). The number of reported cases in the United States has been increasing every year, from 348 cases in 2000 to 4,008 cases in 2018 (Anonymous 2018^c, Anonymous 2019^a). In Arkansas, the number of cases of human granulocytic anaplasmosis is relatively low with a peak of 16 reported cases in 2015 and four reported cases in 2019 (Anonymous 2019^a). Most cases of human granulocytic anaplasmosis are from the north east and upper mid-west with 90% coming from Maine, Rhode Island, Vermont, Minnesota, New Hampshire, Wisconsin, and New York (Anonymous 2019^a). More than 50% of cases are reported in June and July, this is probably because it is peak period of outdoor activity for people and *I. scapularis* are active (Anonymous 2019^a).

The range and time of infection of *A. phagocytophilum* is consistent with the range and activity of *I. scapularis* nymphs, which are the primary vector (Bakken et al. 2005, Davoust et al. 2005). In the United States, *A. phagocytophilum* is maintained in the environment in a zoonotic cycle between *I. scapularis* and their mammalian hosts that act as reservoirs (Goodman et al. 2005). While white-footed mice (*Peromyscus leucopus*) are the most important reservoir, there are many other wildlife species (such as raccoons, squirrel, opossums, and skunks) that can also act as reservoirs (Telford et al. 1996, Levin et al. 2002).

Lyme Disease

Lyme disease was first recognized in 1972 in Connecticut when clustered outbreaks of an unknown disease characterized by a skin lesion and arthritis occurred in the summer and fall. (Steer et al. 1977). While the epidemiology of the disease indicated that it was vectored by an arthropod, the disease-causing agent was not discovered until 1982. Burgorder et al. found spirochetes in an *Ixodes dammini* (renamed *I. scapularis* in 1993) tick from an endemic area in New York that matched those found in patients infected with Lyme disease. The spirochete found was named *Borrelia burgdorferi* and it is the only species known to cause Lyme disease in North America (Stanek et al. 2012). In 2018, Lyme disease was the most common tick-borne disease in the United States with 23,588 reported cases (Anonymous 2018^a). Most Lyme disease cases occur in the Northeast and Midwest with 96% of reported cases coming from 14 states. The disease appears to be expanding; in 2008 the number of counties with more than 10 cases/100,000 people rose from 324 in 2008, to 415 in 2018 (Anonymous 2018^a). Southern states usually have a relatively low number of reported Lyme disease. For example, Arkansas had 4 reported cases in 2019 (Anonymous 2019^b).

Symptoms of Lyme disease usually begin 7 - 10 days after the bite of an infected *I. scapularis* or *Ixodes pacificus* (Cooley and Kohls) (Gerber, 2012). Most patients begin with the development of an erythema migrans and have flu like symptoms, if symptoms are left untreated chronic Lyme disease may develop (Tugwell et al. 1997).

Ixodes scapularis is the main vector of *B. burgdorferi* in the eastern United States and *I. pacificus* is the main vector in the western United States (Gray 1998, Anonymous 2019^c). Most cases of Lyme disease are reported in the summer and fall, this time frame occurs directly after the peak activity period of *I. scapularis* nymphs (Gray 1998). Infected *I. scapularis* and *I.*

pacific ticks usually must feed for 36-48 hours before the spirochetes are transferred to the host (Eldridge and Edman 2000, Vignes et al. 2001). While adult *I. scapularis* ticks have increased rates of infection, they are much larger than nymphs and therefore get removed faster (Eldridge and Edman 2010). *Borrelia burghdorferi* is maintained in the environment through infected ticks' interactions with their wildlife hosts.

The main hosts of adult *I. scapularis* are white-tailed deer, cattle, dogs and other medium to large mammals (Keirans et al. 1996). Hosts used by *I. scapularis* vary within its geographic range, they have been known to parasitize 125 different species of mammals, birds, and lizards (Lane et al. 1991, Spielman et al. 1985). The primary hosts of larvae and nymphs are rodents in the north and reptiles in the south (Apperson et al., 1993). In a study testing the prevalence of *B. burghdorferi* in nymphs, areas where mice are the main host have high infections rates and areas where lizards are the main host have low infections rates (Eldridge and Edman 2000). This could partially explain why southern states have lower rates of Lyme disease despite the abundant white-tailed deer population (Eldridge and Edman 2000). Another explanation for the Lyme disease gradient is behavioral differences between *I. scapularis* nymphs in the North and South. A 2015 study (Arsnoe et al. 2015) observed the differences in questing behavior of *I. scapularis* nymphs collected from one Lyme endemic area (Wisconsin) and two non-endemic areas (North Carolina and South Carolina). Nymphs from the endemic areas are 3.9-19.5 times more likely than non-endemic nymphs to spent time questing above the leaf litter, thus increasing the likelihood they will encounter humans. This behavioral difference is likely due to environmental factors; skinks and lizards (preferred hosts of nymphs in the south) spend much of their time under leaf litter and *I. scapularis* ticks in the south must spend more time under the leaf litter to survive higher air temperatures (Arsnoe et al. 2015) (Diuk-Wasser et al. 2012).

Molecular diagnostics of tick-borne pathogens

In the 1870's, Rocky Mountain spotted fever became one of the first tick-borne diseases to be recognized in the United States. With molecular diagnosis not yet available, the disease was recognized because of its' clustered outbreaks, high fatality rate and economic impact (Harden, 1985). Detection of tick-borne pathogens increased dramatically in the 1980's when polymerase chain reaction (PCR) was invented (Estrada-Pena et al. 2013). The PCR procedure can amplify trace amounts of DNA from biological samples such as tissue or blood. Using PCR, researchers can amplify pathogen DNA present in tick samples before using analysis techniques such as gene sequencing or gel electrophoresis (Richards 2012). It can take longer to identify the tick-borne pathogen responsible for human disease in clinical settings because they often have non-distinguishing symptoms and lower exposure rates (Koopmans 2014). Molecular diagnostic procedures such as PCR rflp, real time PCR and ELISA can detect much lower parasite levels than standard parasitological procedures such as blood smears (Salim et al. 2008) (Adamu et al. 2016). An increase in use of molecular diagnostics in outpatient and clinical settings will amplify our ability to identify novel and recurring disease-causing pathogens in the future (Koopmans 2014).

PCR-RFLP

The polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) procedure uses PCR to duplicate sample DNA with gene specific primers and then cuts the DNA at specified nucleic acid sequences using restriction enzymes (Dahouk et al. 2005). The DNA fragments in the PCR product are then separated by size using procedures such as electrophoresis or mass spectrometry (Dahouk et al. 2005). PCR-RFLP is common for the detection of single pathogen species from tissue samples and is limited mainly by the availability of primers and

supplies (Tijssse-Klasen et al. 2014). The species-specific primers used for pathogen detection are made using known DNA sequences. As a result, primer availability can be biased towards popular research species of the time (Cabezas-Cruz et al. 2018).

An improvement on the PCR-RFLP procedure is the development of multiplex PCR. Using multiplex PCR, two or more primers are used in the same reaction to amplify multiple target sites at once. For multiple primers to be used effectively they must have complimentary annealing temperatures and distinct weights so they can be differentiated while using gel electrophoresis (Henegariu et al. 1997). Multiplex PCR can simultaneously increase the amount of data produced while decreasing the monetary and labor costs of each assay performed (Henegariu et al. 1997).

ELISA

Enzyme-linked immunosorbent assay (ELISA) is an assay uses biological samples to quantify the number of antibodies produced in response to antigen coated into the wells. Readily available supplies and reagents, accuracy and accessibility by numerous laboratories has made ELISA one of the most widely used procedures in hospital and clinical settings for the identification of biological agents. One of the drawbacks to ELISA is the inability to detect antibody presence at the low levels present during the beginning stages of disease (Hosseini et al., 2018). For in-host pathogen detection, DNA-based diagnostics are superior to serological procedures because antibodies are not created until the infection has already established and will persist after the infection has been cured (Chan et al. 2013). DNA from the disease-causing pathogen is present while the pathogen is and does not persist after the infection has been cured.

Tick-borne pathogen studies

In a 2001 Arkansas study, (Eades 2001) cloth sweeps were used to collect ticks from vegetation. After collection, ticks were stored in vials with preservative before running molecular diagnostics for pathogen detection. The ticks were pooled by sex and life stage for lysis and DNA isolation. PCR was then performed with primers 16s+2 and 16s-1, these primers amplify the “a” segment of the 16s tick RNA gene. After PCR was complete, the product was loaded into a 1.5% agarose gel for electrophoresis. If bands appeared on the gel at the 300bp level it indicated that tick DNA was successfully isolated. After successful DNA isolation, similar PCR and electrophoresis methodologies were used with different primers to detect the presence of pathogens. Human monocytic ehrlichiosis was found in a small percentage of the ticks in 1999 and 2000 (.04% and .03%, respectively).

Another Arkansas study (Trout 2010) used PCR as a detection method for pathogens in ticks collected from deer, canines, and felines. After DNA isolation PCR was performed with the 16S rRNA gene primers, and electrophoresis was used to confirm the presence of DNA. If DNA was present, samples were put through similar procedures with genus specific pathogen primers. Of the tested ticks, 32.8% were positive for the *flaB* gene found in *Borrelia* spp.

A Texas study (Sanders 2011) collected serum from 432 feral hogs and used ELISA to determine previous exposure to *Rickettsia*, *Ehrlichia* and *Borrelia* pathogens. The results of the ELISA were 27.59%, 13.18% and 2.12%, respectively, positive for these pathogens. A Kentucky study (Dunlap et al. 2011) collected ticks from two feral hogs and used PCR to test for the presence of *Rickettsia* spp., *E. chaffeensis*, *E. ewingii* and *B. burgdorderi*. Of the 33 ticks tested (31 *A. americanum* and 2 *D. variabilis*), 24 were positive for *Rickettsia* spp., 6 were positive for *E. chaffeensis* and 3 were positive for *E. ewingii*. Another study from Mississippi

(Castellaw et al. 2011) used indirect fluorescent antibody assays to test feral hog blood and found antibodies to *E. chaffeensis*.

A 2015 Arkansas study (Trout Fryxell 2015) used blood samples and 1,731 ticks collected from white-tailed deer and canines to test for the presence of *Rickettsia* species. Blood samples from deer and canine hosts were stored on FTA card and kept at room temperature until testing. PCR procedures were used on the ticks and blood samples to test for the presence of two *Rickettsia* genes, *gltA* and *rompB*. No white-tailed deer, two canines and 29% (502) of the ticks were PCR positive for the *Rickettsia* genes. All five of the tick species collected (*A. americanum*, *I. scapularis*, *A. maculatum*, *R. sanguineus*, *D. variabilis*) were represented in the *Rickettsia* positive samples.

Wildlife as tick hosts

To manage tick-borne diseases, the associated wildlife reservoirs of the pathogens must be identified and managed, as well. The medically important tick species in Arkansas have been reported on a wide range of hosts in the south east United States. It is more useful to look at the hosts used by tick vectors on local scales to understand the risk tick-borne pathogen transmission (McCoy et al. 2013). Many of the surveys done in Arkansas have collected ticks from canids, felines, white-tailed deer, and rodent species. These surveys have found *A. maculatum*, *A. americanum*, *D. variabilis*, *I. scapularis* and *R. sanguineus*; and from these, *B. burgdorferi*, *R. parkeri* and *R. amblyommi* have been found (Trout et al. 2010, Trout et al. 2012, Simpson and Hink 1993). To date, there have been no surveys of the tick species and tick-borne pathogens from the Arkansas feral hog population. These hogs are in every county of Arkansas and could potentially be a reservoir for medically important ticks and tick-borne pathogens.

Feral hogs

Feral hogs, *Sus scrofa* (Erxleben), have been an invasive species in the United States for over 500 years and for centuries their populations were isolated to the south east, California, and Hawaii (Bevins et al., 2014). In 1982, feral hogs were thought to present in a few counties in 17 states (Timmons et al. 2012). As of 2018, feral hogs are present in 38 states and in 43% of all counties in the United States (Anonymous 2018^b). The rapid spread of feral hogs can be attributed to 5 main means of dispersal: 1) intentional movement and/or introduction for recreational hunting, 2) escaped individuals from hunting preserves, 3) escaped individuals from farm operations, 4) intentional release by owners and 5) movement of established feral populations (Gipson et al., 1997). Despite the multiple means of introduction into wild systems, feral hog's biology and life history is what makes them a successful invasive species. Feral hog's rapid reproductive rate and lack of significant predators allows them to take over new habitats quickly and easily (Bevins et al. 2014). Feral hog's primarily consume plant material, but they will also feed on meat, and bird and reptile eggs. Damage caused by feral hogs includes reduction of plant and wildlife biodiversity, extensive agriculture damage, ground water contamination, and pathogen transmission to humans and animals (Bevins et al. 2014, Anonymous 2018^b).

Regarding pathogen transmission, feral hogs can transmit 30 viral and bacterial pathogens and are the host of approximately 40 parasite species (Hutton et al. 2006). In Florida, feral hogs have been found as a host for: *A. americanum*, *Amblyomma auricularium* (Conil), *A. maculatum*, *D. variabilis*, and *I. scapularis* (Allan et al. 2001, Anderson et al. 2018). In Kentucky: *D. variabilis* and *A. americanum* (Dunlap et al. 2011). In Texas: *A. americanum*, *Amblyomma cajennense* (F.), *A. maculatum*, *D. albipictus*, *D. halli*, *D. variabilis* and *I.*

scapularis (Sanders et al. 2013). In New Hampshire: *Dermacentor albipictus* (Musante et al. 2014). In Tennessee: *D. variabilis* and *A. americanum* (Cohen et al. 2010). Most of the ticks collected from feral hogs in these studies were adults, with few nymphs and larvae (Anderson et al. 2018, Sanders et al. 2013).

Feral hogs have been reported in all 75 counties in Arkansas with 21 of these counties having substantial contact. Population densities are dependent on location, habitat type and land management. Damages associated with feral hogs in Arkansas include water quality degradation, agriculture damage and destruction of wildlife habitat and food plots. In 2014, University of Arkansas Cooperative Extension agents were asked to estimate the total monetary losses in their county associated with feral hogs. Of the county agents that responded to the survey, 15% reported losses between \$10,000 – \$50,000, 13% reported losses greater than \$50,000 and one county's losses exceeded \$250,000 (Anonymous 2015). The remaining county agents reported little to no damage. Also, in 2014 10 hunting dogs in Arkansas died from pseudorabies virus after being in contact with feral hogs (Anonymous 2015).

Feral hogs have been found with attached *D. variabilis* and *A. maculatum* ticks in areas outside of those tick's respective ranges, indicating that hogs could be partially responsible for expanding their ranges (Sander 2011). With Arkansas's wide-spread feral hog population, collecting seasonal tick species and tick-borne pathogen samples could identify high risk areas and seasons in the state. Control efforts such as culling reservoir hog populations and tick reduction programs could be focused on high-risk regions and seasons to reduce pathogen circulation most effectively in the environment.

Research Objectives

The objectives of this study were to identify:

- 1) the species and abundance of ticks that parasitize feral hogs (*Sus scrofa* L.) in Arkansas,
- 2) life stages of these ticks, and
- 3) bacterial pathogens found in the ticks of hogs.

Table 1. Diseases associated ixodid tick species in the United States (Anonymous 2019b). Bourbon virus is not included because its host species is not known.

| Tick species | Associated Disease(s) |
|---------------------------------|---|
| <i>Amblyomma americanum</i> | Ehrlichiosis, Heartland virus, STARI (Southern tick-associated rash illness) and Tularemia |
| <i>Amblyomma maculatum</i> | *Rocky Mountain spotted fever |
| <i>Dermacentor andersoni</i> | Rocky Mountain spotted fever, Colorado tick fever and Tularemia |
| <i>Dermacentor occidentalis</i> | 364D rickettsiosis |
| <i>Dermacentor variabilis</i> | Rocky Mountain spotted fever and Tularemia |
| <i>Ixodes cookei</i> | Powassan disease |
| <i>Ixodes pacificus</i> | Anaplasmosis, Lyme disease |
| <i>Ixodes scapularis</i> | Anaplasmosis, Babesiosis, <i>Borrelia mayonii</i> , <i>Borrelia miyamotoi</i> , Lyme disease and Powassan disease |
| <i>Rhipicephalis sanguineus</i> | Rocky Mountain spotted fever |

*Might mean spotted fever rickettsiosis caused by *Rickettsia parkeri*.

Table 2. Diseases associated ixodid tick species in Arkansas (Anonymous 2017^a). Bourbon virus is not included because its host species is unknown.

| Tick Species | Associated Disease(s) |
|---------------------------------|--|
| <i>Amblyomma americanum</i> | ehrlichiosis, tularemia, heartland virus and STARI |
| <i>Amblyomma maculatum</i> | *Rocky Mountain spotted fever |
| <i>Dermacentor variabilis</i> | Rocky Mountain spotted fever, tularemia |
| <i>Ixodes scapularis</i> | Lyme disease, anaplasmosis, babesiosis |
| <i>Rhipicephalus sanguineus</i> | Rocky Mountain spotted fever |

*Might mean spotted fever rickettsiosis caused by *Rickettsia parkeri*.

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Chapter 2: Tick species and life stages collected from Arkansas feral hogs

Abstract

Feral hogs (*Sus scrofa* L.) are found in every Arkansas county and are spreading throughout the southeastern United States. This is a public and animal health concern because feral hogs occupy the same geographical areas as humans and livestock and can harbor over 45 animal diseases and parasites. There are no surveys of the tick species parasitizing the Arkansas feral hog population. Tick collections were taken from feral swine trapped for control purposes by Arkansas Game and Fish Commission, USDA APHIS Wildlife Services and University of Arkansas Cooperative Extension Service personnel. Between February 2019 and January 2020, 3,110 ticks were collected off 220 hogs from 11 Arkansas counties. Four tick species were collected: *Amblyomma americanum* (L.) (95.4%), *Dermacentor variabilis* (Say) (2.4%), *Amblyomma maculatum* (Koch) (0.6%), and *Ixodes scapularis* (L.) (0.3%). Single tick species infestations were the most common with 132 of 138 (95.7%) of these hogs harboring *A. americanum*. The remaining single species infestations were four hogs infested with *I. scapularis* and two with *D. variabilis*. Dual species infestations were seen on 42 hogs with 37 (88.1%) of these being *A. americanum* and *D. variabilis*.

Key words: Ixodidae, Arkansas, feral hogs, *Amblyomma*

Introduction

Feral hogs have been documented as an invasive species in the United States for over 500 years (Bevins et al. 2014). From 1982 – 2018 feral hogs have expanded from 17 states to 38 states and are now present in 43% of counties in the United States (Timmons et al. 2012) (Anonymous 2018). The ability of feral hogs to expand their range so quickly is attributed to

five means of dispersal: migration of existing populations, intentional release by owners, intentional introduction for recreational hunting, escape from hunting preserves and escape from farming operations (Gipson et al. 1997). After establishment, hogs cause a reduction in plant and wildlife biodiversity, ground water contamination, agricultural damage, and pathogen transmission to humans and animals (Bevins et al. 2014) (Anonymous 2018).

Feral hogs are well documented in Arkansas as an invasive species. All 75 Arkansas counties have established hog populations and 21 of these counties have reported substantial numbers (Anonymous 2015). The population densities of feral hogs are variable, depending on land management, location, and habitat type. Impact associated with feral hogs in Arkansas include destruction of wildlife habitat, destruction of food plots, reduction of wildlife biodiversity, water quality degradation and agriculture damage. In a 2014 survey, University of Arkansas Cooperative Extension agents estimated the total monetary losses associated with feral hogs in their county (Anonymous 2015). Most counties (56%) reported no monetary loss or losses between \$1.00 and \$10,000.00. Losses between \$10,000.00 – \$50,000.00 were reported by 15% of county of agents, 13% reported losses greater than \$50,000.00 and one county's losses exceeded \$250,000.00. Monetary losses from feral hogs have been estimated, but the ticks and associated tick-borne pathogens hogs harbor is vastly understudied. The state-wide proximity hogs have to homeowners, animal agriculture, and recreational hunters make this an important field of study. For example, in 2014, 10 Arkansas hunting dogs died from pseudorabies virus after being in contact with feral hogs (Anonymous 2015).

Feral hogs are a host to approximately 40 parasite species, and 30 viral and bacterial pathogens in the United States (Hutton et al. 2006) In Florida, feral hogs have been found as a host for *A. americanum*, *Amblyomma auricularium* (Conil), *A. maculatum*, *D. variabilis*, and *I.*

scapularis (Allan et al. 2001, Merrill et al. 2018); in Kentucky: *D. variabilis* and *A. americanum* (Fritzen et al. 2011); in Texas, *A. americanum*, *Amblyomma cajennense* (F.), *A. maculatum*, *D. albipictus*, *D. halli*, *D. variabilis* and *I. scapularis* (Sanders et al. 2013); in New Hampshire, *Dermacentor albipictus* (Musante et al. 2014); and in Tennessee, *D. variabilis* and *A. americanum* (Cohen et al. 2010). Most of the ticks collected from feral hogs in these studies were adults, with few nymphs and larvae (Merrill et al. 2018, Sanders et al. 2013, Sanders 2011). *Dermacentor variabilis* and *A. maculatum* ticks have been collected from hogs outside of the known range of those tick species (Sander 2011). This indicates that hogs could play a role in expanding ranges of these ticks. With Arkansas's widespread feral hog population, seasonal tick collection could help identify new tick species in the state and identify high risk areas. Tick collections from feral hogs can be used for molecular diagnostic testing for pathogens (Fritzen et al. 2011, Musante et al. 2014, Sanders 2011). The objectives of this study were to survey the species, life stages that seasonality of ticks that parasitize feral hogs in Arkansas.

Materials and Methods

Ticks were collected from February 2019 – January 2020 from 220 feral hogs trapped by Arkansas Game and Fish Commission, USDA APHIS Wildlife Services and University of Arkansas CES personnel. Except for collections taken from Buffalo National River (Permit # BUFF-2018-SCI-0010), all other collections were taken hogs trapped on private property. Collection kits containing vials filled halfway with 95% ethanol, forceps, FDA cards for blood samples, disposable transfer pipettes, cleansing wipes, hand sanitizer, instructions and data collection sheets were assembled and delivered to trappers. Hogs were baited with corn into the corral traps and could feed for multiple days to acclimate to the trap. Corral traps were equipped

with motion detection cameras and an electric gate. Trapping personnel received video footage when hogs were in the corral and closed the corral gate when all or most of a sounder (herd of feral swine) was present.

Ticks were collected from 220 feral hogs in 11 Arkansas counties (Figure 1). Collections were taken by personnel opportunistically from feral hogs being trapped and exterminated for control efforts. Ticks and blood samples were taken from 10 hogs if the sounder had more than 10 individuals and all hogs if the sounder had less than 10 individuals. Before making collections, personnel assigned each hog a number and recorded the date, collection site, size, and sex of hogs. Ticks were collected from the belly, ears, and armpits of the hogs and stored in 20 ml scintillation vial containing 95% ethanol labeled with the hog's number. Blood samples were taken using a heart puncture or hypodermic needle. One-three drops of blood were placed inside the circle on the FTA card using one disposable transfer pipette per hog. FTA cards air-dried on site before being placed in small plastic baggies for transportation and storage for future molecular diagnostics.

Ticks were identified to species, sex, and life stage use morphological features at University of Arkansas Medical and Veterinary Entomology lab. Adult specimens were identified per Kierans & Litwak (1989) and immatures were identified per Kierans & Durden (1998). After identifications, ticks were sorted into separate vials with 95% ethanol by species and life stage and labeled with the hog's identification number. Voucher specimens were deposited in the University of Arkansas Insect Genetics Lab in Fayetteville, AR. Identified ticks were stored at the University of Arkansas Insects Genetics Lab awaiting molecular testing for the presence of *Rickettsia*, *Borrelia*, and/or *Ehrlichia* spp.

Results and Discussion

A total of 3,109 ticks were collected from 220 hogs from 2019-2020. Four tick species were collected from 30 sampling sites in 11 Arkansas counties (Figure 1). Most of the ticks collected were *Amblyomma americanum* (95.4%), followed by *Dermacentor variabilis* (2.4%), *Amblyomma maculatum* (0.6%) and *Ixodes scapularis* (0.3%) (Table 1). Forty ticks were damaged during collection and could not be identified to sex and species. One hundred thirty-eight hogs were infested with a single tick species: 132 with *A. americanum*, four with *I. scapularis*, and two with *D. variabilis* (Table 2). Dual species infestations were present on 42 hogs: 37 hogs were infested *D. variabilis* and *A. americanum*, four hogs with *A. americanum* and *A. maculatum* and one hog with *A. americanum* and *I. scapularis* (Table 2). Tick loads on the hogs ranged from 0 to 96 ticks (median = 112, mean = 14, STDEV = 16). In a few instances, corral conditions were extremely muddy, resulting in conditions that prevented successful tick collections from 40 hogs.

Amblyomma americanum ticks were found January – November, at every collection site and were the only tick species found in larval and nymphal life stages (Table 1) (Figure 1). Lower occurrence of larval and nymphal ticks on feral hogs has been documented in feral hog tick surveys from Texas and Florida (Merrill et al. 2018, Sanders et al. 2013, Schuster 2011). Immature ticks are smaller than adults, and spend less time feeding on hosts, this could partially explain why they are not found as often on feral hogs. The large percentage of *A. americanum* collected is unexpected because while *A. americanum* have been collected in multiple tick surveys from feral hogs, and an Arkansas dog and white-tailed deer survey, they have not accounted for such a large percentage of the collections (Greiner et al. 1984, Sanders et al. 2013, Cohen et al. 2010, Schuster 2011, Trout et al. 2012).

Dermacentor variabilis ticks were collected April – October from nine counties (Polk, Yell, Washington, Searcy, Carroll, Newton, Scott, Crawford, and Logan) (Figure 3).

Amblyomma maculatum adults were found in May 2019 at one collection site in Scott county (Figure 3). *Amblyomma maculatum* have been collected from feral hogs, sometimes in very high numbers, in other tick surveys. Time of year of these collections ranges from spring to fall, depending on the location (Anderson et al., 2018, Goddard, 2007, Schuster, 2011). *Ixodes scapularis* adults were found in November 2019 at one collection site in Crawford county (Figure 3). Previous tick surveys have only found *I. scapularis* ticks on feral hogs in the fall/winter months, and Trout (2010) only found *I. scapularis* ticks on Arkansas dog and white-tailed deer late in the year (Merrill et al. 2018, Greiner et al. 1984, Schuster 2011).

These results support unpublished data from the Arkansas Tick Project that *A. americanum* is the most abundant Ixodid tick in Arkansas (Smith and Loftin 2018, personal comm.). However, most tick collections were made from February – July so these data may not be as representative of species that are more active in the winter months. Arkansas is also in the range of *Dermacentor albipictus* (Packard), which is active in the fall and winter and not represented in these data. These data are a useful starting point in understanding the role feral hogs play as a host of Ixodid tick species in Arkansas, and the southern United States. Further research on this subject should include multi-year sampling, and collection locations in south and eastern Arkansas. More collections taken in the winter months could detect different tick species. However, it is difficult to trap feral hogs in the winter due to interference with hunting seasons and competition with mast crops that make corral baits less attractive to the hogs. Ticks collected for this survey will be used in molecular diagnostic tests for the presence of *Borrelia*, *Ehrlichia* and *Rickettsia* pathogens.

Table 1. Tick species and life stages collected from feral hogs from 2019-2020.

| Species | L | N | M | F | Total |
|----------------------|----------|----------|----------|----------|--------------|
| <i>A. americanum</i> | 3 | 368 | 1209 | 1386 | 2966 |
| <i>A. maculatum</i> | 0 | 0 | 11 | 7 | 18 |
| <i>D. variabilis</i> | 0 | 0 | 22 | 54 | 76 |
| <i>I. scapularis</i> | 0 | 0 | 0 | 9 | 9 |
| *NA | | | | | 40 |

*NA = number of ticks too damaged to identify sex and species

** L = larvae, N = nymphs, M = male adults, F = female adults

Table 2. Summary of single and dual species tick infestations found on feral hogs, 2019-2020.

| Single species infestation | | | | |
|-----------------------------------|----------|----------|----------|----------|
| Total hogs | A | M | D | I |
| 138 | 132 | 0 | 2 | 4 |

| Dual Species Infestation | | | | | | |
|---------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Total hogs | AM | MD | VA | SA | SV | SM |
| 42 | 4 | 0 | 37 | 1 | 0 | 0 |

* A= *A. americanum*, M= *A. maculatum*, V= *D. variabilis*, S= *I. scapularis*

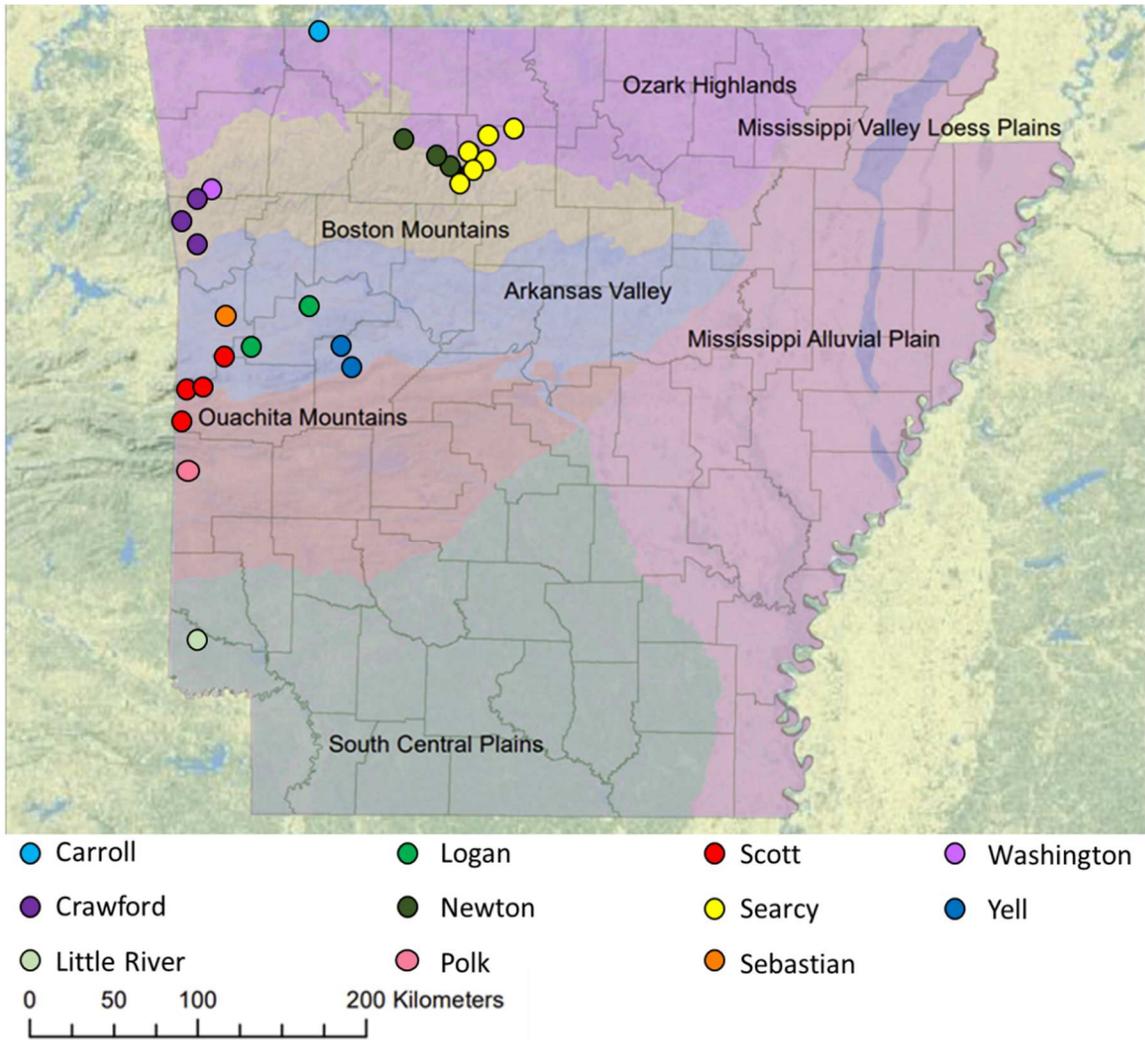


Figure 1. Locations of feral hog traps where ticks were collected, 2019-2020.

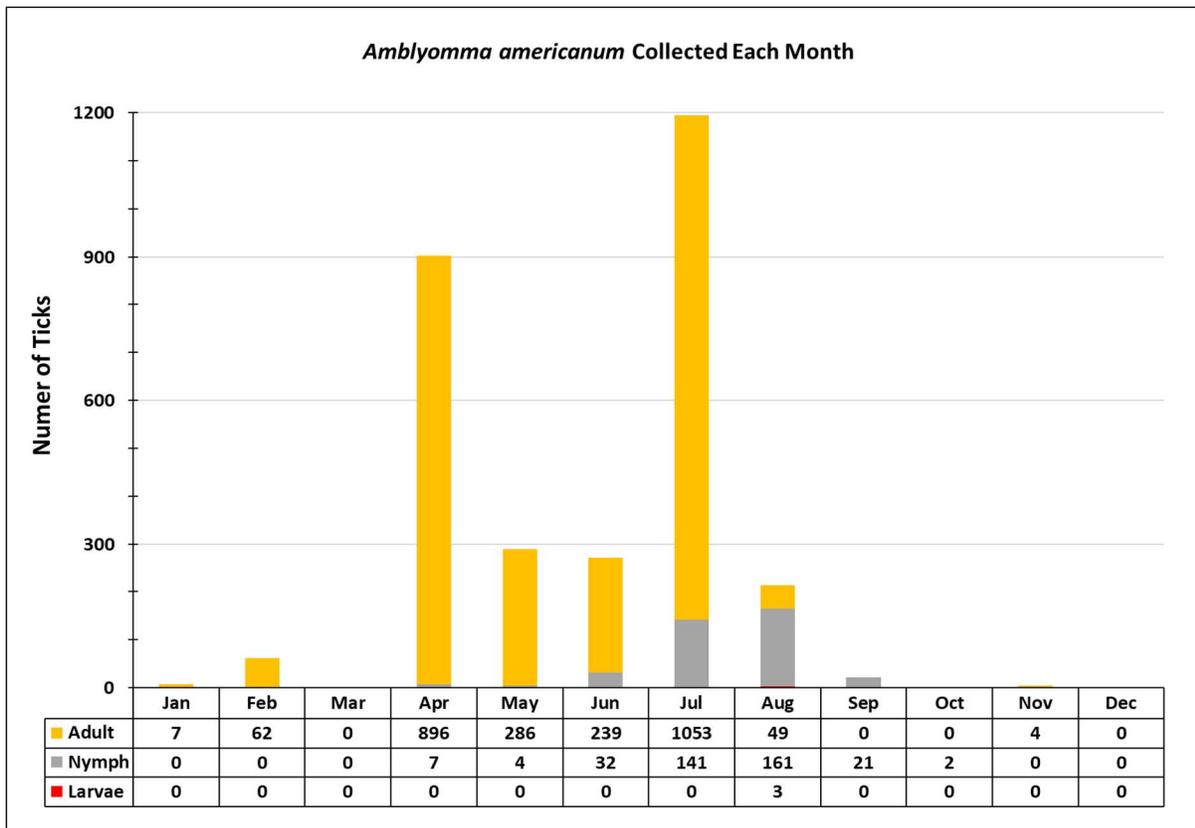


Figure 2. Number of *Amblyomma americanum* ticks collected each month, 2019-2020.

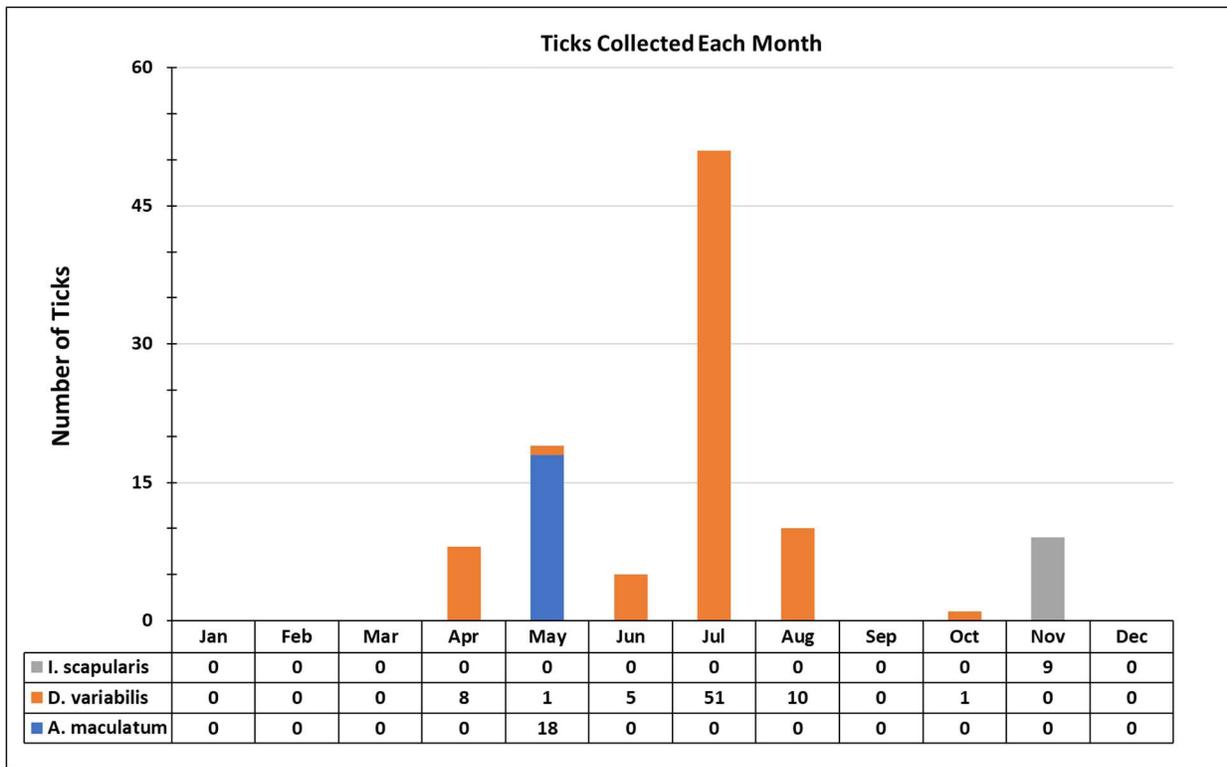


Figure 3. Number of adult *Ixodes scapularis*, *Dermacentor variabilis* and *Amblyomma maculatum* adults collected each month, 2019-2020.

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Chapter 3: Prevalence of tick-borne pathogens in Ixodid ticks collected from Arkansas feral hogs

Abstract

Feral hogs (*Sus scrofa* L.) are found in every county of Arkansas and are found in proximity to humans and livestock. As hogs invade new habitat, they bring ticks and potentially tick-borne pathogens with them. These tick-borne pathogens cause human and livestock diseases such as ehrlichiosis, spotted fever rickettsiosis and Lyme disease. This study used pooled DNA extractions and PCR to survey the *Rickettsia*, *Ehrlichia*, and *Borrelia* bacteria found in ticks collected from Arkansas feral hogs 2019-2020. Every DNA extraction pool contained between 1-10 ticks of the same species, life stage, sex, and host. DNA extractions used in this survey consisted of 72 *Amblyomma americanum*, 11 *Amblyomma maculatum* and nine *Dermacentor variabilis* pools. PCR was performed using primers specific to citrate synthesis gene in *Rickettsia*, 16S gene in *Ehrlichia* and *flab* gene in *Borrelia*. Results of this survey were 24 positive samples for *Rickettsia* spp. bacteria from 23 *A. americanum* adult pools and 1 *D. variabilis* adult pool. None of the samples were positive for *Borrelia* or *Ehrlichia* spp. Five *Rickettsia* positive samples were purified, sequenced and a 100% match for *Rickettsia amblyommatis*. No *Rickettsia rickettsii* was found but survey results show that ticks feeding on feral hogs in Arkansas are hosts of *Rickettsia amblyommatis* pathogens

Keywords: *Amblyomma americanum*, *Dermacentor variabilis*, *Rickettsia*, PCR

Introduction

Feral hogs are a wide-spread and understudied invasive species in Arkansas. They have been reported in all 75 counties in Arkansas with 21 of these counties having substantial contact

(Anonymous 2015). Feral hogs have been reported to host 4 common Ixodid tick species in Arkansas, *Ixodes scapularis* (L.), *Amblyomma americanum* (L.), *Amblyomma maculatum* (Koch), and *Dermacentor variabilis* (Say) (Allan et al. 2001, Merrill et al. 2018, Fritzen et al. 2011, Sanders 2011). The tick – borne diseases that are reported most often are bacterial; rickettsiosis is the most prevalent with over 1,000 reported cases each year from 2017 - 2019 (Anonymous 2019^a). Rickettsiosis is followed by ehrlichiosis (235 cases), tularemia (71 cases), anaplasmosis (4 cases) and Lyme disease (4 cases) (Anonymous 2019^b). The numbers of reported tick-borne diseases have been increasing in Arkansas since 2011, highlighting the need for active monitoring of ticks and their host species (Anonymous 2019^b).

In the United States, Rocky Mountain spotted fever (RMSF) is the most prevalent *Rickettsia* disease (Cohen et al. 2009). Arkansas, Oklahoma, Missouri, North Carolina, and Tennessee account for approximately 60% of Spotted Fever Rickettsiosis cases, although cases have been reported throughout the continental United States (Anonymous 2019^a). In Arkansas, the main vector species is *D. variabilis* (Anonymous 2019^b). *Amblyomma maculatum* infected with *Rickettsia parkeri* have been reported in multiple southern states, indicating that *A. maculatum* could be a vector for Spotted Fever Rickettsiosis in Arkansas (Cohen et al. 2009).

Ehrlichia bacteria can cause ehrlichiosis disease in humans and animals. In the United States, 35% of reported human ehrlichiosis infection cases come from Arkansas, Missouri, and Oklahoma (Anonymous 2017). In 2019, Arkansas had 235 reported cases of human ehrlichiosis (Anonymous 2019^b). All reported cases of human ehrlichiosis in Arkansas are reported under the category of “Ehrlichiosis” without indication of which *Ehrlichia* species caused the disease. Most cases will occur in the spring and summer when the vector of human ehrlichiosis, *Amblyomma americanum* is most active (Fishbein et al. 1994) (Anonymous 2017).

Borrelia bacteria are the causal pathogen of Lyme disease in the United States. *Ixodes scapularis* is the vector of *B. burgdorferi* in the eastern United States and *I. pacificus* is the vector in the western United States (Anonymous 2018^a, Gray 1998). Most cases of Lyme disease are reported in the summer and fall, directly after the peak activity of *I. scapularis* nymphs (Gray 1998). It has been shown that adult *I. scapularis* ticks have higher rates of *B. burgdorferi* infection, but they are larger than nymphs and therefore get removed faster (Eldridge and Edman 2000). The primary host of larvae and nymphs is the white footed mouse (*Peromyscus leucopus*) in the north and reptiles (*Eumeces inexpectatus*, *Scincella lateralis*, *E. laticeps* and *Ophisaurus ventralis*) in the south (Apperson et al. 1993). In a study testing the prevalence of *B. burgdorferi* in nymphs, areas where mice are the main host have high infections rates and areas where lizards are the main host have low infections rates (Eldridge and Edman 2000).

Molecular diagnostics such as PCR and immunochemical tests such as ELISA are used to detect bacterial pathogens in ticks and their hosts. In a 2010 tick borne pathogen survey in Tennessee, 30% of ticks collected from feral hogs were rtPCR positive for *R. amblyommatis* and 1% were positive for *R. montana* Lackman (Moncayo et al. 2010). In a 2011 Texas study, feral hog sera samples were tested with ELISA for previous exposure to *Borrelia*, *Rickettsia* and *Ehrlichia* bacteria (Sanders 2011). One hundred seventeen hogs were positive for previous exposure to *Ehrlichia* spp (13.8%) and 245 were positive for previous exposure to *Rickettsia* spp. (27.6%) (Sanders 2011). Forty-two hogs were positive for multiple pathogens with 30 being *Ehrlichia* and *Rickettsia*, ten for *Rickettsia* and *Borrelia* and one for *Ehrlichia* and *Borrelia* (Sanders 2011). As part of the same study, blood samples from feral hogs were multiplex PCR tested for the presence of the common tick-borne pathogen groups *Borrelia*, *Rickettsia* and

Ehrlichia. Two of 233 (0.85%) of these blood samples were positive for *Borrelia* (Sanders 2011). *Rickettsia* and *Ehrlichia* were not detected (Sanders 2011).

The objective of this study was to conduct a molecular survey of the *Borrelia*, *Ehrlichia* and *Rickettsia* pathogens occurring in ticks collected from feral hogs in Arkansas.

Materials and methods

Tick samples were collected from 220 feral hogs in eleven Arkansas counties from 2019-2020 and preserved in 95% ethanol (Figure 1, Table 1, Table 2). The reduction in tick collections for 2020 was due to COVID-19 restrictions. Most of the tick collections were taken from hogs trapped on private property with the exception a few collections made at Buffalo National River (Permit # BUFF-2018-SCI-0010). Hogs were baited with corn into corral traps and allowed to feed for multiple days to acclimate. Corral traps were equipped with motion detection cameras and an electric gate. Trapping personnel received video footage when hogs were in the corral and remotely closed the corral gate when all or most of a sounder (herd of feral swine) was present.

Adult specimens were identified to species per Kierans & Litwak (1989) and immatures were identified per Kierans & Durden (1998). Voucher specimens were deposited in the University of Arkansas Insect Genetics Lab in Fayetteville, AR. Ticks were pooled for DNA extractions due to the high numbers collected. Pools contained 1-10 ticks from the same host, species, life stage, and sex. A total of 83 pooled DNA extractions were used for this study. Sixty-two of the pooled extractions were *Amblyomma americanum* adults, ten were *A. americanum* nymphs, nine were *Dermacentor variabilis* adults and one was *Amblyomma maculatum* adults.

Before DNA extraction, ticks were cut longitudinally and allowed to dry. DNA was extracted using Puregene technique with in-house reagents (Samwell and Russell 2001). Extracted DNA was resuspended in 50 ul TrisHcl and stored at -20°C until PCR. To confirm the DNA extraction was successful, the first PCR performed was with primer set LepF (5' ATTCAACCAATCATAAAGATATTGG-3') and LepR (5' TAAACTTCTGGATGTCCAAAAAATCA-3') that amplifies the COI region and produces 700 bp amplicon (Footit et al. 2008).

Once tick DNA was confirmed, PCR was used again to identify the presence of *Borrelia*, *Ehrlichia* and *Rickettsia* pathogens. *Borrelia* was screened using primers FLaLL (5'-ACATATCTTATCATTCTAATAGC-3') and FLaRL (5'-GCAATCATAGCCATTGCAGATTGT-3') that amplify the *flab* gene (Barbour et al. 1994, Bacon et al. 2003) Thermocycler conditions for screening *Borrelia* are as follows: denature at 95°C for 2 minutes followed by 40 cycles of 94°C for 45 seconds, 55°C for 30 seconds, 72°C for 45 seconds, and a final extension of 72°C for 5 minutes. Successful amplification produces a 300bp amplicon.

Ehrlichia was screened using primers HE1F (5'-CAATTGCTTATAATTTGGTTATAAAT-3') and HE#R (5'-TATAGGTACCGTCTTATCTTCCCTAT-3') (Anderson et al. 1992). Thermocycler settings for *Ehrlichia* are as follows: denature at 88°C for one minute followed by 37 cycles of 88°C for one minute, 55°C for one minute-thirty seconds, 72°C for one minute, and a final extension of 72°C for 5 minutes. Successful amplification produces a 389bp amplicon.

Rickettsia was screened using primers RrCS.372 (5'-TTTGTAGCTCTTCTCATCCTATGGC-3') and RrCS.989 (5'-

CCCAAGTTCCTTTAATACTTCTTTGC -3') that target the *Rickettsia* specific citrate synthesis gene (Kollars and Kengluocha 2001). Thermocycler settings for *Rickettsia* are as follows: denature at 95°C for 2 minutes followed by 40 cycles of 94°C for one minute, 58°C for one minute, 72 C for one minute, and a final extension of 72°C for 5 minutes. Successful amplification produces a 617bp amplicon.

Each PCR product was subjected to electrophoresis in a 2% agarose gel (Owl Separation Systems, LLC), stained with ethidium bromide and visualized with UV light (BioDocit™ Systems, UVP, LLC, Upland, CA) to confirm amplification. Positive controls were used for bacteria (*Rickettsia rickettsii* genomic DNA ATCC® number NR-48826 from BEI Resources, *Ehrlichia chaffeensis* genomic DNA ATCC® number NR-50091 from BEI Resources and *Borrelia burgdorferi* genomic DNA number 35210D-5 from ATCC®) and negative controls using distilled water were used. Five *Rickettsia* positive samples were purified and sequenced using Eurofins Genomics (Diatherix, Huntsville, AL) for species confirmation using reference sequences from GenBank (National Center for Biotechnology Information). These positive samples' sequences were visualized, and a BLAST (National Center for Biotechnology Information) search was performed to compare genetic sequences to samples in GenBank.

Results and Discussion

DNA extraction and amplification using LepF/LepR primers was attempted on 406 samples with 85 successful extractions (21%). From the positive samples, 13 were from an individual tick and 72 were pooled DNA extractions. *Rickettsia* DNA was identified in 24 pooled extractions, 23 were *A. americanum* adults and one was a *Dermacentor variabilis* adult (Figure 1, Table 3). Positive samples came from Carroll, Crawford, Logan, Newton, Polk, Scott, and Searcy counties. From the positive 23 *A. americanum* pooled samples, 19 were females and

four were males (82.6%) (Table 4). The disparity between males and females could be due to larger blood meals needed by adult females to lay eggs. Collections that resulted in positive *Rickettsia* ticks occurred in February, April, May, June, and July (Table 2). This is consistent with *A. americanum*'s life cycle and adult activity periods (Bouzek et al. 2013). Adults have also been collected as early as February in Mississippi (Jackson et al. 1996).

Five pooled *Rickettsia* positive *A. americanum* extractions were purified, sequenced, and ran through a GenBank BLAST search. All five positive extractions were a 100% match to *Rickettsia amblyommatis* (accession number KY273595), a *Rickettsia* spp. that causes mild symptoms compared to *R. rickettsii* (Snellgrove et al. 2021). *Rickettsia amblyommatis* has been found previously in ticks collected from feral hogs in Tennessee (Moncayo et al. 2010). The prevalence of *Rickettsia* spp. and *R. amblyommatis* is also consistent with a 2015 Arkansas tick survey (Trout Fryxell 2015). This survey collected ticks from white-tailed deer and canines and found 244 *A. americanum* and seven *D. variabilis* ticks PCR positive for the *gltA*, *rompB* and *ompA* genes found in *Rickettsia* pathogens (Billingsley 2015). None of the sequenced products were matches to *R. rickettsii*, and the most common sequence matches for *A. americanum* was *R. amblyommatis*.

Borrelia and *Ehrlichia* DNA was not detected in this survey. A 2001 tick-borne pathogen survey from Arkansas collected ticks from vegetation and detected a small percentage (0.04%) of ticks positive for *Ehrlichia* pathogens (Eades 2001). *Ehrlichia* spp. and *Rickettsia* spp. have been reported from a feral hog tick-borne pathogen survey in Kentucky (Fritzen et al. 2011). In this study, 32 *A. americanum* and two *D. variabilis* ticks were collected from two feral hogs. Eleven of these ticks tested PCR positive for either *Rickettsia* spp., *E. chaffeensis* or *E.*

ewingii (Fritzen et al. 2011). In a 2010 Arkansas tick-borne pathogen survey (Trout Fryxell 2012), DNA extractions and PCR were performed on ticks collected from deer and canines.

The *flaB* gene present in *Borrelia* spp. pathogens was detected in 27.5% (583 of 2123) of tested DNA extractions (Trout Fryxell 2012). *FlaB* positive extractions by tick species was 252 *I. scapularis*, 50 *A. maculatum*, 88 *R. sanguineus*, 161 *A. americanum* and 19 *D. variabilis*. Half of the *flaB* positive samples for each tick species were sequenced and the majority matched *Borrelia lonestari* Barbour (Trout Fryxell 2012). While *Borrelia* pathogens have been detected in Arkansas, it was expected that *Borrelia burgdorferi* would not be found in this survey because all DNA extractions were from *A. americanum*, *A. maculatum* and *D. variabilis* ticks from feral hogs. A small number of *I. scapularis* ticks were collected from the sampled feral hogs, but the DNA extractions were unsuccessful.

Five of 23 *Rickettsia* positive samples were sequenced and a match to *Rickettsia amblyommatis*. Continuing this study, more ticks collected from the feral hogs should be processed to get a more complete picture of the pathogens present. It is possible that some of the unprocessed ticks could be infected with *Ehrlichia* or *Borrelia* spp. Also, all the *Rickettsia* positives should be sequenced to get a better view of the species present. One of the unsequenced positive samples was from *D. variabilis*. *Dermacentor variabilis* is an accepted vector of the *Rickettsiae* responsible for Rocky Mountain spotted fever, *Rickettsia rickettsii*.

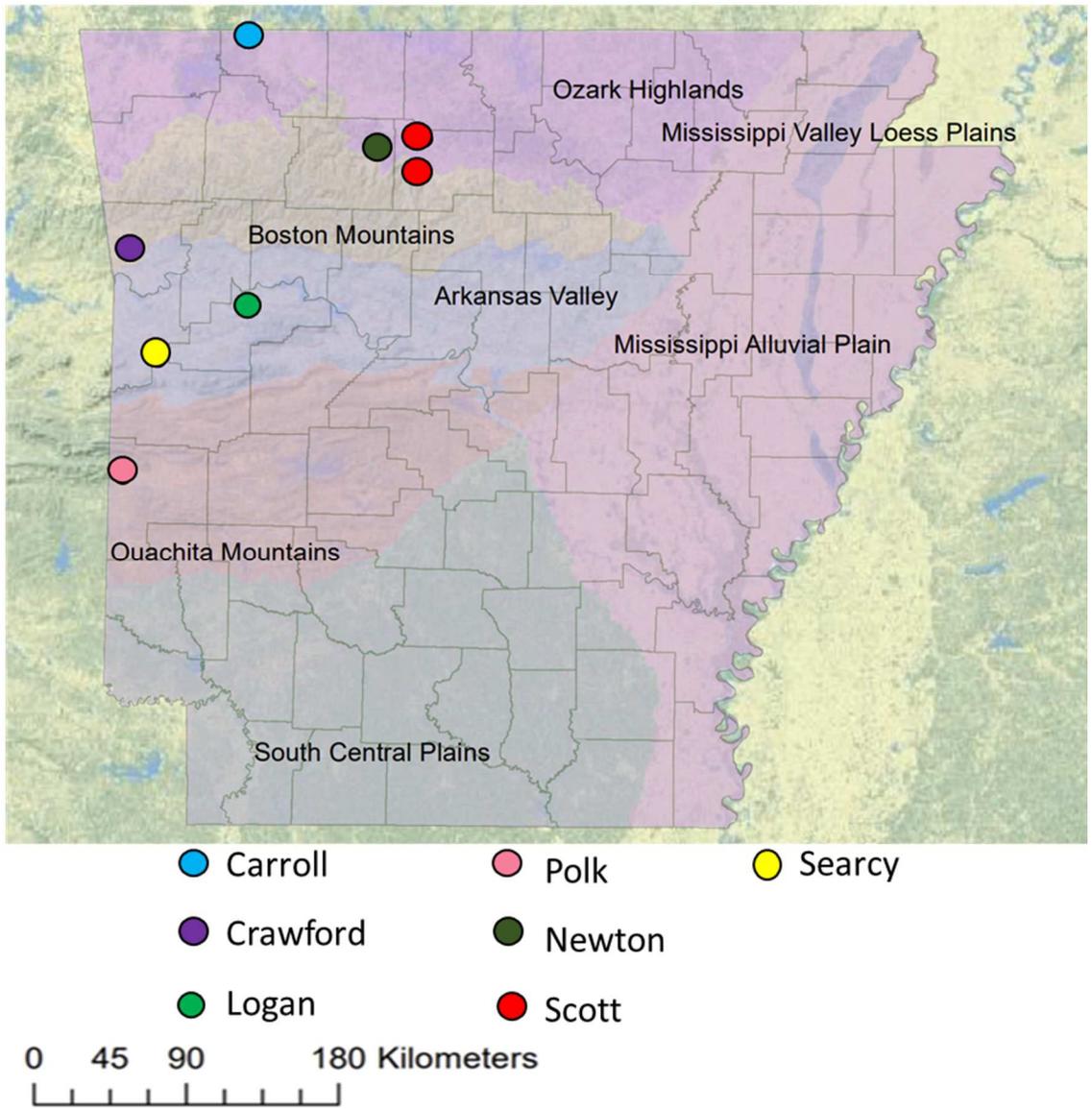


Figure 1. Locations of feral hog traps where ticks were collected, 2019-2020.

Table 1. Weight and sex of feral hogs used for tick collections.

| County | <50 lbs. | | >50 lbs. | |
|--------------|----------|----|----------|----|
| | M | F | M | F |
| Carroll | - | 2 | 3 | 5 |
| Crawford | 9 | 4 | 9 | 14 |
| Little River | 1 | 2 | 1 | 5 |
| Logan | - | 1 | 8 | 6 |
| Newton | 5 | 12 | 3 | 4 |
| Polk | 4 | 1 | 4 | 9 |
| Scott | 6 | 4 | 8 | 26 |
| Searcy | 4 | 13 | 6 | 16 |
| Sebastian | - | - | 2 | 3 |
| Washington | - | - | 3 | - |
| Yell | - | 3 | 7 | 7 |
| Total | 29 | 42 | 54 | 95 |

Table 2. Tick collection dates and counties.

| Date | County |
|------------------|---------------|
| *28-II-2019 | Crawford |
| *12-IV-2019 | Polk |
| *12-IV-2019 | Scott |
| 12-IV-2019 | Yell |
| *15-IV-2019 | Searcy |
| *4-V-2019 | Logan |
| 11-V-2019 | Sebastian |
| 20-V-2019 | Washington |
| 24-V-2019 | Scott |
| *14-VI-2019 | Searcy |
| *3-VII-2019 | Carroll |
| *3-VII-2019 | Newton |
| 3-VII-2019 | Newton |
| 10-VII-2019 | Newton |
| 10-VII-2019 | Searcy |
| 23-VII-2019 | Searcy |
| 12-VIII- 2019 | Scott |
| 12-VIII- 2019 | Yell |
| 25-VIII- 2019 | Crawford |
| 30-VIII- 2019 | Crawford |
| 26-IX-2019 | Little River |
| 8-X-2019 | Logan |
| 21-XI-2019 | Scott |
| 26-XI-2019 | Crawford |
| 2-I-2020 | Polk |
| 9-I-2020 | Scott |

*Indicates a collection site that resulted in a positive *Rickettsia* sample.

Table 3. Prevalence of *Rickettsia*, *Ehrlichia* and *Borrelia* in ticks from Arkansas feral hog by species and sex.

| Tick species | Sex | <i>Rickettsia</i> , no. positive (%) | <i>Ehrlichia</i> , no. positive (%) | <i>Borrelia</i> , no. positive (%) |
|----------------------|-----|--------------------------------------|-------------------------------------|------------------------------------|
| <i>A. americanum</i> | | 23 (0.31) | 0/ (0.00) | 0 (0.00) |
| | M | 4 (0.17) | - | - |
| | F | 19 (0.83) | - | - |
| <i>D. variabilis</i> | | 1 (0.10) | 0 (0.00) | 0 (0.00) |
| | M | 0 (0.00) | - | - |
| | F | 1 (1.00) | - | - |
| <i>A. maculatum</i> | | 0 (0.11) | 0 (0.00) | 0 (0.00) |
| | M | - | - | - |
| | F | 0 (0.00) | 0 (0.00) | 0 (0.00) |

Table 4. Prevalence of *Rickettsia* in ticks from Arkansas feral hog by county and sex.

| County | <i>Rickettsia</i> positive samples | Sex | |
|---------------|---|------------|---|
| Carroll | 1 | M | - |
| | | F | 1 |
| Crawford | 5 | M | 3 |
| | | F | 2 |
| Logan | 3 | M | - |
| | | F | 3 |
| Newton | 1 | M | - |
| | | F | 1 |
| Polk | 3 | M | - |
| | | F | 3 |
| Scott | 6 | M | 1 |
| | | F | 5 |
| Searcy | 5 | M | - |
| | | F | 5 |

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Chapter 4: Conclusions

Feral hogs are widespread in Arkansas and the southern United States, often being found near humans and livestock (Anonymous 2015). This proximity makes it crucial that we are aware of the ticks and tick-borne pathogens they carry to minimize risk. Data obtained in this thesis provides a starting point for understanding the role that feral hogs play in the tick-borne pathogen transmission cycle. *Rickettsia* bacteria and multiple tick species were found from feral hogs in this study. Continuing this research, more tick collections and pathogen surveillance should be conducted in additional Arkansas counties. The following sections provide summaries of results and discussion about future recommendations for feral hog and tick-borne pathogen surveillance in Arkansas.

Tick species and life stages collected from feral hogs

From February 2019 to January 2020, 220 feral hogs were sampled from 11 Arkansas counties. In total, 3,110 ticks of four species were collected. *Amblyomma americanum* was the most common species collected, accounting for 96.6% of the collected ticks. The remaining species found were *Dermacentor variabilis*, *Amblyomma maculatum* and *Ixodes scapularis*. Most ticks collected were adults. *Amblyomma americanum* was the only species collected in immature stages and found in every sampled county.

From the 220 sampled hogs, 138 were infested with a single tick species and 42 were infested with two tick species. *Amblyomma americanum* accounted for 132 of the single species infestations followed by four hogs infested with *I. scapularis* and two with *D. variabilis*. For the hogs with dual species infestations, the most common was *A. americanum* and *D. variabilis*. Dual species infestations are concerning because both tick species are known vectors of at least

one disease causing pathogen and simultaneous feeding could increase the pathogen load of the hogs.

Most of the tick collections were taken from trapping on private property done for control purposes. Understanding the tick species and life stages that parasitize feral hogs is important because of the great potential for interactions between hogs, humans, and livestock. It is important for the landowners to be aware of the tick-borne pathogen risks associated with feral hog to better protect themselves and their livestock. Continuing to collect ticks from feral hogs on private property will provide a more accurate account of all the tick species that may be present. It would also be beneficial to survey livestock found on the same property as feral hogs to see if they are carrying the same tick species.

Rickettsia* found in *Amblyomma americanum* and *Dermacentor variabilis

Ticks from every sampled hog were PCR tested for *Rickettsia*, *Ehrlichia* and *Borrelia* spp. From the 85 pooled DNA extractions that were tested, 24 were positive for *Rickettsia* spp. citrate synthesis gene (Kollars and Kengluocha 2001). No *Ehrlichia* or *Borrelia* pathogens were found. *Amblyomma americanum* adults accounted for 23 of the 24 positive samples, with the final positive being a *Dermacentor variabilis* adult. From the positive *A. americanum* extractions, 19 of these were females. Female adults may make up a large percentage of the positives because they require larger blood meals to lay eggs. Five of the *Rickettsia* positive samples were sequenced and found to be a 100% match to *Rickettsia amblyommatis* on a GenBank BLAST search. *Rickettsia amblyommatis* is a pathogenic bacterium that causes mild spotted fever symptoms when compared to *Rickettsia rickettsii* (Snellgrove et al. 2021).

Finding 24 *Rickettsia* spp. positives and *Rickettsia amblyommatis* in all five of the sequenced samples merits additional research on these samples. The remaining *Rickettsia* positives should be sequenced to find if there are other *Rickettsia* species present. Specifically, the *Dermacentor variabilis* adult female that was positive should be sequenced. *Dermacentor variabilis* is a known vector of *Rickettsia rickettsii*, the pathogen responsible for causing Rocky Mountain spotted fever. Additionally, more ticks collected from the feral hogs should be PCR tested. Due to the high volume of ticks and time limitations, not all the sampled ticks were able to be tested.

Final thoughts

My thesis was a survey of the tick species and life stages of the Arkansas feral hog population, and the pathogens carried by these ticks. Feral hogs were sampled from 11 counties in the Northwest, Northcentral, West, and Southwest regions of Arkansas. Further studies on the parasite and pathogen loads of feral hogs in the state should be conducted. More immediate studies should focus on the main human pathogens found in these ticks (*Borrelia*, *Ehrlichia* and *Rickettsia*) because these bacteria already cause diseases in Arkansas (Anonymous 2019). After baseline knowledge of these bacteria in relation to feral hogs is developed, further epidemiology studies should focus on other pathogens and parasites they may be carrying. This research would be specifically important to regions of Arkansas with large feral hog populations, or areas where feral hog hunting is popular. Close contact with feral hogs can lead to pathogen transmission, for example, ten hunting dogs died in 2014 after contact with feral hogs (Anonymous 2015). A more comprehensive knowledge of feral hogs' vector status will help protect Arkansas residents and landowners from potential disease. Additionally, tickborne pathogen surveillance should be performed in Arkansas using ticks collected from humans and domestic animals. This is important because Arkansas has tickborne diseases, including 1,065 cases of Spotted fever

rickettsiosis and 169 cases of *Ehrlichia chaffeensis* infection in 2018 (Anonymous 2018^a, Anonymous 2018^b).

My thesis research provides insight to the role that feral hogs play in the tick-borne pathogen transmission cycle. However, additional surveys need to be conducted to get the full picture. It is important to continue research on the tick and tickborne pathogen loads of feral hogs in Arkansas.

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