Rickettsial Agents Detected in Ixodid Ticks (Acari: Ixodidae) Collected from *Sus scrofa* (Artiodactyla: Suidae) in Florida and South Carolina[1]

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Abstract Feral swine, *Sus scrofa* L., have become a nuisance to landowners across the United States by damaging agriculture, property, and ecosystems. Additionally, these animals have been found to host various ixodid ticks including *Amblyomma americanum* (L.), *Amblyomma maculatum* Koch, *Dermacentor variabilis* (Say), and *Ixodes scapularis* Say, which can maintain and transmit several rickettsial pathogens to livestock, wildlife, and humans. Though previous research has identified the maintenance cycle of several rickettsial pathogens in ticks and native wildlife, little is known about the role *S. scrofa* plays in supporting ixodid ticks and the pathogens these ticks could be harboring. This study sought to identify rickettsial agents (Rickettsiales: Anaplasmataceae and Rickettsiaceae) in ticks collected from *S. scrofa* obtained in Florida and South Carolina. Overall, ticks from four species (*A. americanum*, *D. variabilis*, *I. scapularis*, and *A. maculatum*) totaling 258 collected individuals were obtained from *S. scrofa* ($n = 45$). We found an *Ehrlichia chaffeensis* Anderson et al. infection prevalence in *A. americanum* of 2.7% and 2.9% in Florida and South Carolina, respectively. A *Rickettsia parkeri* Lackman et al. prevalence of 100% and 33% was found in *A. maculatum* from Florida and South Carolina, respectively. Additionally, a 0.9% infection prevalence of *R. parkeri* was identified in *A. americanum* collected in South Carolina. A 1.9% *Ehrlichia ewingii* Anderson et al. infection prevalence was documented in collected *A. americanum* in South Carolina. Further studies are warranted to better understand the role *S. scrofa* plays in the natural maintenance of rickettsial agents in various regions of the United States.

Key Words *Amblyomma americanum, Dermacentor variabilis*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Rickettsia parkeri*

Feral swine (*Sus scrofa* L.) are an invasive species to the United States. As early as the 16th Century, imported domestic swine were released and European boars were introduced for hunting, which led to these animals interbreeding and development of a feral swine population that eventually occupied a large portion of the country. The range of this species has expanded dramatically in the past few

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decades, encompassing most regions of the country due to its ability to adapt to many different habitat types and to its high reproductive capacity (Gipson et al. 1998, 1999; Mayer and Brisbin 1991; Merrill et al. 2018; Sanders et al. 2013). Additionally, in the United States, *S. scrofa* have been acknowledged in the devastation of crops along with destruction of real property across the country (Pimental et al. 2005). Population management of these nuisance animals by hunters and landowners has been utilized. However, management techniques may increase potential human exposure to ectoparasites on these animals. Some of these ectoparasites, such as ixodid ticks, may be able to harbor and transmit rickettsial pathogens to unsuspecting humans (Sonenshine and Roe 2014). Importantly, the USDA, Animal and Plant Health Inspection Service (APHIS), Wildlife Services maintains a national feral swine damage management program to ensure protection of agricultural resources and domestic animal health. It is critical to our understanding of emerging tick-borne diseases in the United States to examine feral swine populations and associated tick species within the extensive regions that have been invaded by this mammalian species and its continued expansion into new areas (Musante et al. 2014).

Previous studies have documented several tick species found on *S. scrofa* in the southcentral and southeastern United States including *Amblyomma americanum* (L.), *Amblyomma maculatum* Koch, *Amblyomma mixtum* Koch, *Dermacentor variabilis* (Say), and *Ixodes scapularis* Say. A study conducted in Texas from 2008 to 2011 collected the following tick species from harvested feral pigs ($n = 806$): *Amblyomma cajennense* sensu lato (F.) (62% infestation rate [IR]), *D. variabilis* (13% IR), and *A. maculatum* (13% IR) (Sanders et al. 2013). The *A. cajennense* collected in this study are likely *A. mixtum* according to a recent taxonomic study indicating that *A. cajennense* collected in the United States from older literature likely refers to *A. mixtum* (Nava et al. 2014). Another study recorded *A. mixtum* (48% IR), *D. variabilis* (22% IR), and *A. maculatum* (19% IR) collected from feral swine ($n = 81$) in Texas during September 2015 (Corn et al. 2016). Several studies conducted in Florida documented *A. americanum*, *A. maculatum*, and *D. variabilis* as the most frequently collected tick species obtained from *S. scrofa* (Allan et al. 2001; Greiner et al. 1984; Hertz et al. 2017; Merrill et al. 2018). Additionally, feral swine ($n = 266$) obtained in Tennessee were examined for ectoparasites and the only tick species collected was *D. variabilis* (Henry and Conley et al. 1970).

Some of these ixodid ticks are known to vector several rickettsial pathogens (Rickettsiales: Anaplasmataceae and Rickettsiaceae) such as *Ehrlichia chaffeensis* Anderson et al., *Anaplasma phagocytophilum* Dumler et al. (Foggie), *Rickettsia parkeri* Lackman et al., and *Rickettsia rickettsii* Brumpt (Wolbach) in the United States. Several studies in European countries, including France, Italy, and Spain, have identified *Rickettsia slovaca* Sekeyova et al. at various prevalences (15.7%, 32.1%, and 17.7%, respectively) in *Dermacentor marginatus* Sulzer collected from wild boar, albeit lower numbers of this arthropod were obtained in these studies (Ortuno et al. 2006; Sanogo et al. 2003; Selmi et al. 2009). Despite these previous studies, limited information is known regarding rickettsial pathogen prevalence found in ticks collected from feral swine in the southeastern United States. The objective of this study focused on identifying ticks and any rickettsial agents found in ticks collected from *S. scrofa* trapped in the southeastern United States.

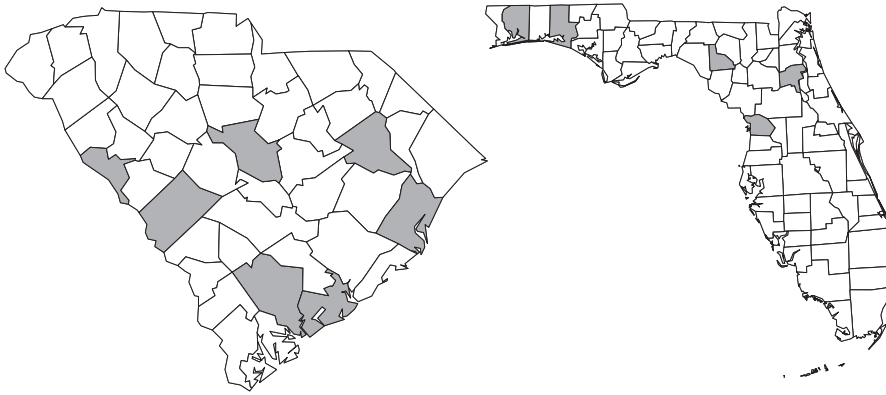

Fig. 1. Maps of South Carolina (left) and Florida (right) indicating counties (shaded) where ixodid ticks were collected from trapped *Sus scrofa* during this study. Note: No scale associated with either state map.

Materials and Methods

Specimen collection. Feral swine were collected from sites in Florida and South Carolina through the National Feral Swine Damage Management Program led by Wildlife Services under the USDA, APHIS. Wildlife Services operates under the guidance of Wildlife Services Directive 2.320 in fulfillment of Executive Order 13112 (1999), which allows Wildlife Services to provide control of invasive species including feral swine. Collection sites were in five counties from Florida and seven counties from South Carolina, with all specimens collected during the months from February to December in 2016 (Fig. 1). Ticks were manually removed from harvested feral swine and placed into 70% ethanol for transport to the laboratory. Tick specimens were identified to species by standard taxonomic keys (Clifford et al. 1961; Cooley 1938; Durden and Keirans 1996; Keirans and Durden 1998) and sent to Centers for Disease Control and Prevention for identification confirmation and rickettsial organism detection.

Extraction of DNA. Tick specimens were at various levels of engorgement; therefore, only half of each specimen was utilized in the DNA extraction process to prevent the DNA extraction membrane from becoming overloaded with tick DNA. Tick specimens were minced by using a sterile scalpel, and then DNA was extracted according to the manufacturer's protocol using a Qiagen DNeasy extraction kit (Qiagen, Valencia, CA). A 1-h lysis/digestion period was utilized during the extraction process. All tick specimen DNA samples were extracted individually and stored at 4°C until screened for rickettsial agents.

Rickettsial testing. Extracted DNA samples from tick specimens were screened for members of the Anaplasmataceae family by using a real-time PCR SYBR Green assay that targets a portion of the 16S rRNA gene as previously described (Allerdice et al. 2017; Eremeeva et al. 2007; Li et al. 2002). Samples were tested in duplicate with a positive control consisting of *Ehrlichia chaffeensis* DNA and a no-template control consisting of molecular grade water. Specimens were considered

positive if the melting temperature was within the expected target range (75.0–80.0°C) and had a PCR cycle threshold (Ct) value less than 40 cycles. Sequence analysis of amplicons from the *groESL* heat shock operon were utilized as previously described (Nicholson et al. 1999) to determine bacterial species identity for any positive specimens screening positive for a member of the Anaplasmataceae family. Tick DNA samples also were screened for members of the *Rickettsia* genus by using a real-time Taqman® PCR assay that targets a 74-base-pair amplicon of the citrate synthase gene as previously described (Stenos et al. 2005). Samples found to have *Rickettsia* DNA were tested with a *Rickettsia amblyommatis* species-specific Taqman PCR assay targeting a 142-base-pair amplicon from the *omp*B gene as previously described (Jiang et al. 2010). Tick DNA samples testing positive for a member of the *Rickettsia* genus also were tested with a *Rickettsia rickettsii*-specific and a *Rickettsia parkeri*-specific Taqman PCR multiplex assay targeting a portion of a hypothetical protein gene and the *omp*B gene, respectively (Denison et al. 2014). The DNA samples that employed the *Rickettsia* spp., *R. amblyommatis, R. rickettsii,* and *R. parkeri* Taqman PCR assays were tested in duplicate with a positive control consisting of *Rickettsia conorii*, *R. amblyommatis*, *R. rickettsii*, or *R. parkeri* DNA, respectively. These samples also were tested with a no-template control consisting of molecular grade water. Samples tested with Taqman PCR assays were considered positive if the sample had a PCR Ct value less than 40 cycles. All real-time PCR reactions were monitored in real time by a Bio-Rad CFX96 Real-Time System (BioRad, Hercules, CA). Samples that were DNA positive for a *Rickettsia* species, yet were not identified using the Taqman PCR assays mentioned above, were identified by sequence analysis of part of the *omp*A 190 kDa protein gene region (Regnery et al. 1991). All sequences were assembled using Geneious 11.1.2 software and analyzed by an National Center for Bioltechnology Information Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) search for matches.

Results

***Sus scrofa* collections.** A total of 45 *S. scrofa* were trapped from Florida ($n = 22$) and South Carolina ($n = 23$). Collections were made from five counties in Florida (Citrus, Lafayette, Putnam, Santa Rosa, and Walton Co.) and seven counties in South Carolina (Aiken, Charleston, Colleton, Florence, Georgetown, McCormick, and Richland Co.) (Fig. 1). Traps were employed each month from February through December in 2016, with documented trapped animals each month except June, October, and December.

Ticks infesting *Sus scrofa*. Overall, ticks collected from *S. scrofa* included *A. americanum* (adults = 201, nymphs = 14), adult *D. variabilis* ($n = 32$), adult *I. scapularis* ($n = 7$), and adult *A. maculatum* ($n = 4$). Ticks were collected at various levels of engorgement. All trapped *S. scrofa* were infested with at least one tick species. The infestation rate and mean tick intensity of each tick species on *S. scrofa* was similar in both states (Table 1). The total mean tick intensity on *S. scrofa* between both states was 5.7 ticks per animal (range of 1–14 ticks). The seasonality of ticks found on *S. scrofa* in Florida and South Carolina documented *A. americanum* being collected from February to September, with the most ticks

Table 1. Overall infestation rate (IR), mean intensity (MI), and number (No.) of *Sus scrofa* infested with ixodid tick species.

	Florida			South Carolina		
Tick species	**IR (%)**	**MI**	**No.**	**IR (%)**	**MI**	**No.**
A. americanum	91.0	5.5	20	82.6	5.5	19
D. variabilis	31.8	2.4	7	26.1	2.5	6
A. maculatum	4.6	1.0	1	13.0	1.0	3
I. scapularis	9.1	3.0	2	4.4	1.0	1

collected in May. *Dermacentor variabilis* were collected during the months of April to September, with most ticks being collected in May (Fig. 2).

Screening for rickettsial organisms. Testing of the collected ticks detected an *Ehrlichia chaffeensis* prevalence of 2.7% ($n = 3$; 95% confidence interval [CI]: 0.9–7.7) and 2.9% ($n = 3$; CI: 1.0–8.1) in *A. americanum* adults from Florida and South Carolina, respectively (Tables 2, 3). The collected ticks infected with *E. chaffeensis* were collected between March and May. An *Ehrlichia ewingii* Anderson et al. infection prevalence of 1.9% ($n = 2$; CI: 0.5–6.7) was identified in adult *A. americanum* ticks collected in South Carolina in May (Table 3). The prevalence of *Rickettsia parkeri* found in *A. maculatum* was 100% ($n = 1$; CI: 20.7–100) and 33.3% ($n = 1$; CI: 6.2–79.2) in Florida and South Carolina, respectively. A low *R. parkeri* infection prevalence of 0.9% ($n = 1$; CI: 0.2–5.2) was also detected in adult *A. americanum* from South Carolina (Table 3). Detection of the putatively nonpathogenic rickettsial agent, *Rickettsia amblyommatis* Karpathy et al., was found at a prevalence of 57.3% ($n = 63$; CI: 47.9–66.1) and 80.0% ($n = 84$; CI: 71.4–86.5) in *A. americanum* adults collected from Florida and South Carolina, respectively. Additionally, infection prevalence of *R. amblyommatis* of 35.0% ($n = 6$; CI: 17.3–58.7) and 66.7% ($n = 10$; CI: 41.7–84.8) was detected in *D. variabilis* collected from Florida and South Carolina, respectively (Table 2 and 3). *Rickettsia rhipicephali* Weiss and Moulder (Burgdorfer et al.) prevalence of 0.9% ($n = 1$; CI: 0.2–5.0) and 11.8% ($n = 2$; CI: 3.3–34.3) was found in Florida in adult *A. americanum* and *D. variabilis*, respectively, while a *Rickettsia buchneri* Kurtti et al. prevalence of 100% ($n = 6$; CI: 61.0–100) was documented in *I. scapularis* from Florida. Two adult *A. americanum* tested positive for both *E. ewingii* and *R. amblyommatis* DNA collected in South Carolina and one *A. americanum* adult tested positive for both *E. chaffeensis* and *R. amblyommatis* DNA collected in Florida, indicating coinfection in these ticks by these agents.

Discussion

The current study identified four tick species that coincide with species found questing on vegetation and infesting *S. scrofa* in previous research in the southeastern United States (Apperson et al. 2008; Burgdorfer 1975; Henry and Conley 1970; Hertz et al. 2017; Merrill et al. 2018). To no surprise, *A. americanum*

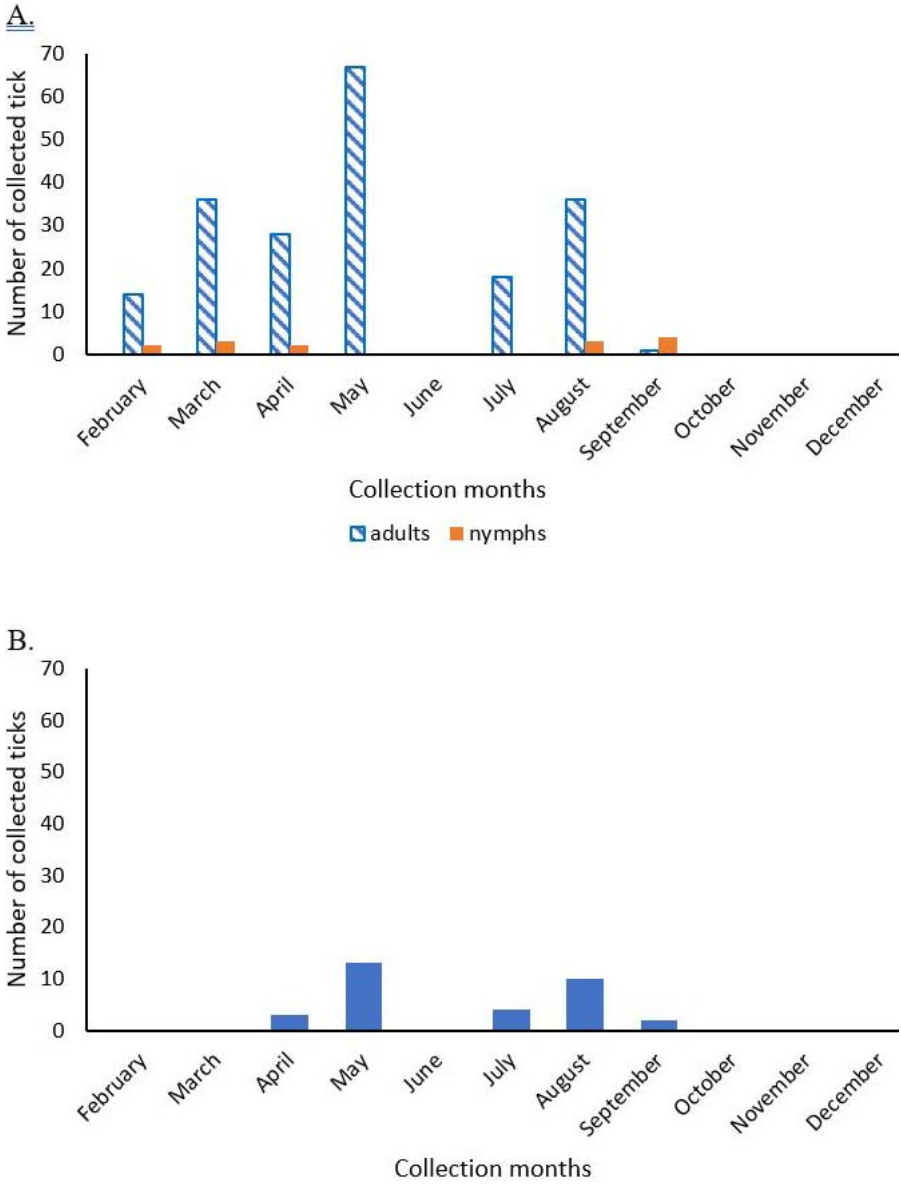

Fig. 2. Overall seasonality of *Amblyomma americanum* (A) and *Dermacentor variabilis* (B) collected from trapped *Sus scrofa* in Florida and South Carolina. Note: No *Sus scrofa* were collected in June, October, and December.

Table 2. Rickettsial agent prevalence percentage (number of positive ticks) in ticks from *S. scrofa* in Florida.

Tick Species	No. of Ticks Tested	*E. chaffeensis*	*E. ewingii*	*R. parkeri*	*R. amblyommatis*
A. americanum	110	2.7 (3)	0 (0)	0 (0)	57.3 (63)
D. variabilis	17	0 (0)	0 (0)	0 (0)	35 (6)
A. maculatum	1	0 (0)	0 (0)	100 (1)	0 (0)
I. scapularis	6	0 (0)	0 (0)	0 (0)	0 (0)
Total overall	134	2.2 (3)	0 (0)	0.8 (1)	51.5 (69)

was the tick species most commonly found on *S. scrofa*, as all life stages have been found to parasitize many host species (Yabsley 2010). The seasonality of *A. americanum* found on *S. scrofa* was similar to other studies in that we found *A. americanum* adults most often from April to June (Jackson et al. 1996; Kollars et al. 2000). *Amblyomma maculatum*, *D. variabilis*, and *I. scapularis* were not found as abundantly infesting *S. scrofa* in this study as in other studies; however, this could be due to the smaller feral swine sample size in both states (Allan et al. 2001; Greiner et al. 1984). *Dermacentor variabilis* was found infesting fewer animals and at a lower mean density on infested animals than was *A. americanum*.

Previously, studies have tested ticks collected from *S. scrofa* in the United States for rickettsial agents and resulted in limited positive findings. One study found rickettsial DNA in putative *Amblyomma triste* Koch ticks associated with *S. scrofa* in Texas, but researchers were unable to identify to species the rickettsial agent found in these specimens (Kubala 2012). Regarding rickettsial pathogen infection prevalence in questing ticks, a study found an overall infection prevalence of 4.7% and 3.5% in *A. americanum* for *E. chaffeensis* and *E. ewingii*, respectively, in questing ticks collected in nine southeastern states (Mixson et al. 2006).

Table 3. Rickettsial agent prevalence percentage (number of positive ticks) in ticks from *S. scrofa* in South Carolina.

Tick Species	No. of Ticks Tested	*E. chaffeensis*	*E. ewingii*	*R. parkeri*	*R. amblyommatis*
A. americanum	105	2.9 (3)	1.9 (2)	0.9 (1)	80.0 (84)
D. variabilis	15	0 (0)	0 (0)	0 (0)	66.7 (10)
A. maculatum	3	0 (0)	0 (0)	33.3 (1)	0 (0)
I. scapularis	1	0 (0)	0 (0)	0 (0)	0 (0)
Total overall	124	2.4 (3)	1.6 (2)	1.6 (2)	75.8 (94)

Additionally, other studies conducted in several southeastern states identified an *R. parkeri* infection prevalence ranging from 8–43% in questing ticks (Paddock et al. 2010; Sumner et al. 2007; Varela-Stokes et al. 2011; Wright et al. 2011). In the current study, *Ehrlichia chaffeensis* DNA was documented in *A. americanum*, which is known as the primary vector of this pathogen in the southeastern United States (Yabsley 2010). A similar infection prevalence for *E. chaffeensis* was found in ticks from both states during this study, which coincides with previous findings in questing ticks. *Rickettsia parkeri* DNA was found at relatively higher prevalence during the current study in *A. maculatum*, a primary vector of this pathogen. However, consideration of the limited number of *A. maculatum* collected during this study should be noted prior to further prevalence analysis. *Rickettsia parkeri* infection also was found in *A. americanum*, albeit one sample, which also has been documented in studies from several southeastern states in questing ticks (Cohen et al. 2009; Goddard and Norment 1986; Paddock et al. 2010; Wright et al. 2015). *Ehrlichia ewingii* was detected in *A. americanum*, a vector of this pathogen, at an infection prevalence similar to previous studies looking at pathogen prevalence in ticks collected from vegetation (Anziani et al. 1990; Mixson et al. 2006; Steiert and Gilfoy 2002). Furthermore, in the current study, coinfection was seen in adult *A. americanum*, as these ticks may have acquired either *E. chaffeensis* and *R. parkeri* or *E. ewingii* and *R. parkeri* infections through their bloodmeal as an adult or during an immature life stage. An agent not known to be pathogenic to humans, *R. amblyommatis*, was found in a large portion of *A. americanum* collected during this study from both states. The high prevalence found in both states is similar to previous studies documenting *R. amblyommatis* in questing *A. americanum* (Apperson et al. 2008; Jiang et al. 2010; Mixson et al. 2006). Additionally, this organism does not appear to be tick-specific, as it has been shown to infect both questing and host-associated *D. variabilis* in this and other studies (Moncayo et al. 2010). The endosymbiont, *Rickettsia buchneri*, was found in *I. scapularis*; likewise, this agent has been commonly found in the United States in *I. scapularis* found on vegetation and attached to humans (Gleim et al. 2019; Tokarz et al. 2019). Few *I. scapularis* were collected during this study and should be taken into consideration when comparing to other findings regarding this organism. *Rickettsia rhipicephali*, usually found in *Rhipicephalus sanguineus* (Latreille) ticks, was detected in *A. americanum* and *D. variabilis* during this study. This agent has been detected in questing *Dermacentor occidentalis* Marx from California and an *Amblyomma* sp. haplotype Nazare collected from birds in Brazil (Philip et al. 1981; Zeringota et al. 2017). This finding only suggests that *R. rhipicephali* can occasionally be acquired by *A. americanum* and *D. variabilis*, but is not evidence that these arthropod species play a role in the natural cycle of this rickettsial agent.

The ecological and molecular data documented in this study indicate the potential for feral swine to be involved in the natural maintenance cycle of *E. chaffeensis* and *E. ewingii* in the southeastern United States where the primary vector for these agents, *A. americanum*, is prevalent. *Rickettsia amblyommatis* was found in a large portion of the *A. americanum* collected from *S. scrofa* in this study; however, due to *R. amblyommatis* being an endosymbiont of *A. americanum*, and its ability to be maintained through transstadial and transovarial transmission, it is likely not utilizing feral swine as a reservoir host (Karpathy et al. 2016).

All of the ticks were obtained with some level of blood engorgement, and results represent a mixture of tick tissues and blood from the swine host. While this demonstrates either native infection of the ticks or acquisition during the feeding, all rickettsial organisms might not be maintained transstadially in nymphs or transovarially in adults. It would be advantageous for further studies to not only look at ticks collected from feral swine, but to also analyze various tissue sample types from the infested swine to better understand the role of this invasive mammalian species in the natural ecology of rickettsial pathogens. This study provides contemporary data on the infection of ticks from feral swine with rickettsial pathogens and other rickettsial organisms. While we had access only to pigs from two states, collaborations with state and federal efforts could facilitate further investigations. The dynamics of rickettsial infections, their interactions, and the role of feral swine as a transport, bloodmeal, and rickettsial amplification host should be examined in future studies.

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