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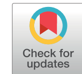
Original article

Presence of diverse *Rickettsia* spp. and absence of *Borrelia burgdorferi* sensu lato in ticks in an East Texas forest with reduced tick density associated with controlled burns



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ABSTRACT

As tick-borne diseases continue to emerge across the United States, there is need for a better understanding of the tick and pathogen communities in the southern states and of habitat features that influence transmission risk. We surveyed questing and on-host ticks in pine-dominated forests with various fire management regimes in the Sam Houston National Forest, a popular recreation area near Houston, Texas. Four linear transects were established-two with a history of controlled burns, and two unburned. Systematic drag sampling yielded 112 ticks from two species, *Ixodes scapularis* (*n* = 73) and *Amblyomma americanum* (*n* = 39), with an additional 106 questing ticks collected opportunistically from drag cloth operators. There was a significant difference in systematically-collected questing tick density between unburned (15 and 18 ticks/1000 m^2) and burned (2 and 4 ticks/1000 m^2) transects. We captured 106 rodents and found 74 ticks on the rodents, predominantly *Dermacentor variabilis*. One unburned transect had significantly more ticks per mammal than any of the other three transects. DNA of *Rickettsia* species was detected in 146/292 on and off-host ticks, including the 'Rickettsial endosymbiont of *I. scapularis*' and *Rickettsia amblyommatis*, which are of uncertain pathogenicity to humans. *Borrelia lonestari* was detected in one *A. americanum*, while *Borrelia burgdorferi* sensu stricto, the agent of Lyme disease, was not detected in any tick samples. Neither *Borrelia* nor *Rickettsia* spp. were detected in any of the mammal ear biopsies (n = 64) or blood samples (n = 100) tested via PCR. This study documents a high prevalence in ticks of *Rickettsia* spp. thought to be endosymbionts, a low prevalence of relapsing fever group *Borrelia* in ticks, and a lack of detection of Lyme disease-group *Borrelia* in both ticks and mammals in an east Texas forested recreation area. Additionally, we observed low questing tick density in areas with a history of controlled burns. These results expand knowledge of tick-borne disease ecology in east Texas which can aid in directing future investigative, modeling, and management efforts.

1. Introduction

Tick-borne diseases have been increasing in number and scope in the United States over the last four decades. Lyme disease, caused by *Borrelia burgdorferi* sensu stricto (s.s.), was first discovered in the 1970s and over the span of twenty years expanded to become the most common vector-borne disease in the United States. From 2004–2016, the number of annual reports of tick-borne bacterial and protozoal diseases more than doubled (Rosenberg et al., 2018). Within the last decade, several tick-borne pathogens have emerged as agents of disease in the United States for the first time, including *Ehrlichia muris eauclairensis*, *Rickettsia parkeri*, *Borrelia miyamotoi*, Heartland virus, deer tick virus (Powassan virus lineage II), and Bourbon virus (Dantas-Torres et al., 2012; Krause et al., 2013; McMullan et al., 2012; Parola et al., 2009; Stromdahl and Hickling, 2012; Wormser and Pritt, 2015). In Texas, where human tick-borne disease is diagnosed less frequently than in some areas of the United States, public and medical awareness of local tick species and pathogens is insufficient (Mitchell et al., 2016; Stromdahl and Hickling, 2012; Williamson et al., 2010).

Amblyomma americanum, the lone star tick, is the most common human-biting tick in the southern United States (Childs and Paddock, 2003). This tick is increasingly recognized as an important vector of several human pathogens (Childs and Paddock, 2003), but not of *B. burgdorferi* s.s. (Stromdahl et al., 2018). Additionally, *A. americanum* is

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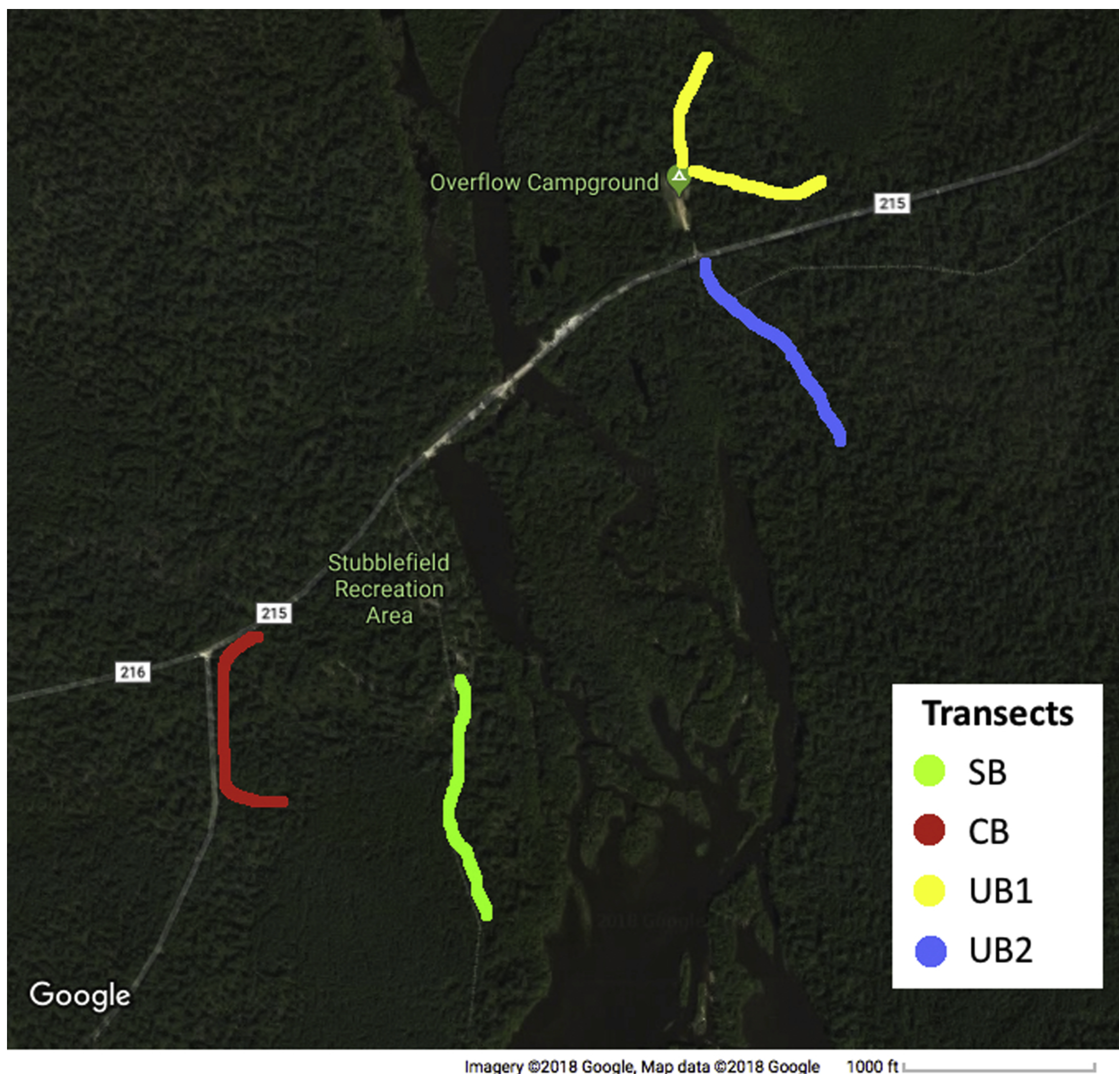

Fig. 1. Satellite map of the study site around Sam Houston National Forest Stubblefield Recreation Area in east Texas, with the four transects indicated by colored lines as described in the legend. SB = sustained burn, CB = ceased burn, UB1 = unburned 1, UB2 = unburned 2. UB1 and UB2 are unburned transects, while CB and SB have been burned.

associated with the development of red meat allergy (Childs and Paddock, 2003; Commins and Platts-Mills, 2013).

Ixodes scapularis, the key vector of *B. burgdorferi* s.s. in the eastern United States, is found across the southeast and in eastern Texas, but *B. burgdorferi* s.s. prevalence in ticks, animals, and humans is generally low in this region (Bowman et al., 2009; Little et al., 2014; Mitchell et al., 2016; Stromdahl and Hickling, 2012; Teltow et al., 1991; Williamson et al., 2010; Yancey et al., 2014). This low prevalence is potentially explained by several hypotheses, including differences in nymphal phenology (Ogden et al., 2018), questing behavior (Arsnoe et al., 2019, 2015), and host feeding preferences (Kollars et al., 1999; Durden et al., 2002), but has been the subject of some controversy, which can confound public health education efforts (Norris et al., 2014).

Other ixodid ticks that feed on humans in Texas include *Dermacentor variabilis*, *Rhipicephalus sanguineus* sensu lato, *Amblyomma cajennense* sensu lato, and *Amblyomma maculatum* (Mitchell et al., 2016; Williamson et al., 2010). In order to inform public health interventions and education, it is necessary to understand which tick species and life stages are active in the local area, which pathogens they are likely to carry, and which reservoir host species are important in the maintenance of the pathogens in nature (Eisen et al., 2012).

In addition to the ticks, mammalian reservoirs, and pathogens, environmental factors must also be considered in characterizing transmission cycles and risk of exposure to tick-borne disease agents. Vegetation has been shown to play a role in tick density, and one proposed strategy to reduce tick populations and pathogen transmission risk is the use of prescribed burns to reduce vegetation density. Previous studies investigating the effect of prescribed burns or wildfire on the abundance of ticks fail to reach consensus on the direction and magnitude of effect, likely due to variation in fire intensity, burn intervals, and possibly tick species examined (Allan, 2009; Davidson et al., 1994; Fisher and Wilkinson, 2005; Gleim et al., 2014; MacDonald et al., 2018; Padgett et al., 2009; Stafford et al., 1998; Wilson, 1986). Fewer published studies have directly investigated the effect of controlled burns on tick-borne pathogen prevalence or risk, with one reporting reduced tick abundance in burn areas but unchanged risk of encountering infected nymphs (Mather et al., 1993), while another more recent paper reports no difference in tick pathogen prevalence between burned and unburned sites, but lower encounter rates with infected ticks at sites subjected to long-term burning (Gleim et al., 2019). The objective of this study was to survey for questing tick density and *Borrelia* and *Rickettsia* spp. in ticks and mammals in a region of east Texas which contains sites with different controlled burn histories.

2. Methods

2.1. Field site

The Sam Houston National Forest (SHNF) in the Piney Woods ecoregion of East Texas comprises 65,979 ha within Montgomery, Walker, and San Jacinto counties, and is intermingled with privately owned residential and agricultural properties. The Stubblefield Lake Recreation Area is located within the SHNF in Walker County along Stubblefield Lake, an oxbow lake of Lake Conroe, 89 km north of Houston, Texas, the largest city in Texas and the 5th most populated metropolitan area in the United States. The area is frequently used for camping, boating, and hiking, and the popular Lone Star Hiking Trail passes through the recreation area. Management of the SNHF is based on the multiple-use concept with uses such as recreation, fish and wildlife, timber, grazing, soil and water, and minerals (USFS, 2018). Many of the forest compartments have prescribed fires as a tool for ecosystem management to reduce heavy accumulations of forest fuels to minimize damages in the event of wildfires and to improve threatened and endangered habitat for wildlife. Burns are managed to clear the undergrowth and deadfall, but preserve the large trees and canopy.

The forest surrounding the recreation area and the nearby overflow campground site is composed of three distinct burn compartments: (i) the 'sustained burn' compartment (SB) has a history of controlled burns every 1–2 years since at least 1993 with the most recent burn in 2014; (ii) the 'ceased burn' compartment (CB) was burned regularly 5 times between 1993 and 2008 but not since; and (iii) the 'unburned' compartment (UB) has no documented history of prescribed burn since at least 1993. One 400 m linear transect was established within each of the burned compartments (SB, CB) and two transects were established within the unburned compartment (UB1, UB2) for a total of four transects (Fig. 1). All four transects are classified as 'Loblolly Pine' forests by the Forest Service and contain primarily pine trees with a mixture of deciduous trees such as oak (*Quercus* sp.), elm (*Ulmus* sp.), and sweetgum (*Liquidambar* sp.). The transects in the unburned forest compartments have more downed woody debris and a much thicker understory compared to the transects receiving prescribed fires. Dominant understory plants in all of the transects include the dwarf palmetto (*Sabal minor*), yaupon holly (*Ilex vomitoria*), and American beautyberry (*Callicarpa americana*), but these are much thicker and more mature in the transects with no fire treatment. Ticks and mammals were sampled along each transect during five site visits from March to April 2015 to capture the spring peak of questing adult *I. scapularis* activity in the south (Dubie et al., 2018; Goltz and Goddard, 2013; Kollars et al., 1999).

2.2. Questing tick collection

At each visit, all four transects were sampled for questing ticks by drag sampling, which consists of dragging a white cloth over vegetation to collect host-seeking ticks (Falco and Fish, 1992). A 1 m^2 white corduroy cloth fixed to a wooden handle was dragged behind or alongside the operator for an average coverage of 575 m^2 for each transect and visit. The cloth was examined every 10–20 m for the presence of ticks, which were collected with forceps and stored in 70% ethanol. Human drag cloth operators and (on three visits) an accompanying canine were also inspected and any ticks present after each transect drag effort were collected into separate vials. These ticks were counted and used for pathogen surveillance but were not included in the analysis for systematic drag sampling. Drag sampling was performed during morning or late afternoon hours. In the laboratory, ticks were identified to species and life stage using a dichotomous key (Sonenshine, 1979). Indices of tick abundance were calculated as the number of ticks of each species and stage collected per 1000 m^2 and per hour of drag sampling.

2.3. Small mammal trapping

Small mammals were trapped along each of the four transects using Sherman live traps (H.B. Sherman Traps, Tallahassee, FL, USA). Forty traps were set along each transect, spaced 10 m apart and baited with sunflower seed for one trap night during each visit. Capture success was calculated as the number of mammals captured divided by the number of effective trap nights, where the total number of trap nights was reduced by the number of empty, tripped traps as follows: number traps set − 0.5 × number tripped traps, assuming that, on average, tripped traps were open for half the night (Hamer et al., 2012; Nelson and Clark, 1973). The species and sex of the captured animals were identified by visual inspection. Each animal was weighed and inspected for ticks, focusing search effort around the ears, face, and back of the neck. A 2 mm diameter punch biopsy was taken from each ear, blood (up to 20 uL) was collected from the tip of the tail, and animals were marked with uniquely-numbered metal ear tags (National Band and Tag Company, Newport, KY, USA). A single ear biopsy was taken from animals recaptured from previous site visits. Ticks and ear biopsies were stored separately in 70% ethanol, and whole blood was frozen prior to analysis. Isofluorane (Sigma-Aldrich, St Louis, MO, USA) was used for anesthesia as needed for some individuals. All animals were released at the site of capture. Wildlife procedures were approved by the Texas A&M University's Animal Care and Use Committee permit # 2012-100, Texas Parks and Wildlife Department Scientific Research Permit # SPR-0512-917, and Special Use Permit SAM010801 from the US Department of Agriculture, Forest Service.

2.4. Molecular methods

DNA extraction was performed on ticks, ear biopsies, and blood using a commercially available kit (E.Z.N.A Tissue DNA Kit; Omega Bio-Tek, Norcross, GA, USA) according to manufacturer's instructions, but with a final elution volume of 60 μL. Prior to extraction, ticks were macerated with scalpels that were flame sterilized between each tick. The DNA from adult and nymphal ticks was extracted individually, whereas larval ticks removed from mammals were pooled for extraction when there was more than one larva of the same species on the mammal at the same time. Pools contained up to 7 larvae of the same species removed from the same host animal on the same date. One ear biopsy per capture was selected for extraction.

Visual tick identification was confirmed on a subset of ticks using a PCR for the 12S rRNA gene (Beati and Keirans, 2001). All tick, and a subset of ear and blood samples were screened for *Rickettsia* via PCR using the primers RrCS 372 and RrCS 989 targeting a 617-bp product of a partial region of the citrate synthase (gltA) gene (Williamson et al., 2010). Confirmatory testing on a subset of *A. americanum* and all *I. scapularis* samples that tested positive on the initial assay was performed through the amplification of a 632-bp region in the *ompA* gene (Zhang et al., 2006). Positive controls (DNA of *Rickettsia amblyommatis* from field-collected *A. americanum*) and negative controls (PCR-grade water) were included in all reactions. The tick, ear, and blood samples were then subjected to two PCRs for *Borrelia*. The first was a nested PCR to amplify a 500–1000 bp region of the 16 S–23 S rRNA intergenic spacer (IGS) of *Borrelia* species (Bunikis et al., 2004). This PCR was repeated for samples that tested positive to control against contamination. Samples that generated a band on either attempt of the IGS PCR were then subjected to a multiplex qPCR with Lyme disease group and relapsing fever group probes to amplify the 16S rRNA gene of *Borrelia* (Tsao et al., 2004). Positive controls of *Borrelia* were from field-collected samples of *B. miyamotoi* or *Borrelia lonestari*. Conventional PCR products were visualized using gel electrophoresis, then amplicons were purified (ExoSAP-IT; Affymetrix, Santa Clara, CA, USA) and sequenced using Sanger sequencing at Eton Bioscience Inc (San Diego, CA, USA). Sequences were visualized in 4Peaks and aligned in MEGA7, then compared to published sequences using the basic local alignment

search tool (BLAST) in GenBank (Altschul et al., 1990). Sequences generated in this study were deposited to GenBank (accession numbers MK882590-97).

2.5. Statistical analysis

To analyze counts of off-host (questing) and on-host ticks on burned and unburned transects, we used general mixed models assuming a negative binomial error distribution as in other studies (Castellanos et al., 2016). We used a zero-inflated model (ZI) when the fit improved (i.e. lower Akaike information criteria). All models were implemented in R (v. 3.3.0) using the package glmmADMB (v. 0.8.3.3). Visit date was included in the models as a random intercept and sampling effort (effective trap nights or meters dragged) per transect was included using the offset function. Significance of all treatment coefficients was assessed through a log-likelihood ratio test of nested models assuming a chi-squared-distribution. ANOVA was used to compare the mean number of ticks per rodent captured across individual transects. Chi-squared tests were used to compare pathogen prevalence in ticks on burned and unburned transects.

3. Results

3.1. Questing ticks

A total of 11,100 m^2 (861 min) of systematic drag sampling was conducted during the study period, ranging from 1800 to 2900 m^2 (110 to 235 min) during each visit. Drag sampling yielded 112 ticks from two species collected from drag cloths: *Ixodes scapularis* ($n = 69$ adults and 4 nymphs) and *A. americanum* ($n = 29$ adults and 10 nymphs). An additional 106 ticks (62 adult and 1 nymphal *I. scapularis*, 26 adult and 17 nymphal *A. americanum*) were collected from persons or the companion dog during drag sampling efforts. These non-systematically-collected ticks were excluded from the statistical models of questing tick density but were included in molecular analyses for pathogen detection. Across all transects, drag-collected tick density was 6/1000 m^2 for *I. scapularis* adults, < 1/1000 m^2 for *I. scapularis* nymphs, 3/1000 m^2 for *A. americanum* adults and 1/1000 m^2 for *A. americanum* nymphs. The total questing tick density was estimated by the model to be five-fold higher (OR = 5.36; $p < 0.001$) in unburned (15 and 18 ticks/1000 m^2) compared to burned (2 and 4 ticks/1000 m^2) transects. The UB1 transect had the most *I. scapularis* ticks collected via drag sampling, with a density of 13 ticks/1000 m^2 or 9 ticks/hour. *Amblyomma americanum* were most dense on UB2, with 8 ticks/1000 m^2 or 6 ticks/hour (Fig. 2). Of the ticks found on humans or the dog for which transect of origin was known, 52 originated from the UB1 or UB2 transects, while none were from CB or SB transects; transect of origin was unknown for 41 ticks.

3.2. Mammals

We had 106 small mammal captures (including 26 recaptures) over an estimated 784.5 effective trap nights, comprising 80 individual small rodents of four genera (Table 1). *Peromyscus* sp. (not identified to species in the field; either *Peromyscus leucopus* or *Peromyscus gossypinus* (Schmidly and Bradley, 2016)) were most commonly captured (n = 69, 86%), followed by *Reithrodontomys fulvescens* (n = 5, 6.3%), *Sigmodon hispidus* (n = 5, 6.3%), and a single *Microtus* sp. (n = 1, 1.2%). Overall capture success averaged 13.5%, ranging from 0 to 40% for any individual transect visit, and generally increasing with each successive visit over the course of the spring. There was no significant difference in capture success between the burned and unburned transects as groups ($p = 0.63$, but the transects along hiking trails (CB and UB2) had lower capture success than the other transects ($p = 0.021$).

Ticks collected from rodents were larval (n = 20 pools representing 43 individual larvae) or nymphal (n = 37) *D. variabilis* and nymphal *I. scapularis* (n = 2). The zero-inflated model predicted a seven-fold increase in the number of on-host ticks collected per effective trap night on unburned relative to burned transects ($p = 0.004$). In particular, one unburned transect (UB1) had significantly more ticks per rodent captured (Table 1) than any of the other three transects (means of 1.5 ticks/rodent versus 0.13 to 0.47 ticks/rodent; $p = 0.005$).

3.3. Molecular testing

All collected ticks (on-host and questing) were subjected to molecular testing for *Rickettsia* and *Borrelia*. Of the 218 questing ticks tested via PCR for *Rickettsia* spp., 142 generated *Rickettsia*-specific amplicons on the gltA screening PCR (Table 2, Fig. 3): 98 *I. scapularis*, and 44 *A. americanum*. A subset of positives (n = 77) were run on the second *Rickettsia* PCR targeting OmpA, and 57 generated amplicons with good quality sequences. Of the 60 tick samples collected from rodents that were tested via PCR for *Rickettsia* spp. (Table 3), 1 *D. variabilis* nymph generated a *Rickettsia*-specific amplicon. All gltA (GenBank MK882590, isolate 15SHT-008) and OmpA (GenBank MK882597, isolate 15SHT-067) sequences from *A. americanum* ticks matched GenBank entries for *Rickettsia amblyommatis* (also known as *Rickettsia amblyommii*; GenBank accession numbers KY273595, JN378401, MF188911). In *I. scapularis* ticks, we detected at least 3 different sequences matching unnamed *Rickettsia* species within the spotted fever group (Fig. 3). The most commonly detected gltA sequence (GenBank MK882591, isolate 15SHT-018) exhibited 100% identity to several isolates of "*Rickettsia* endosymbiont of *I. scapularis*" (GenBank KY678090-93). Amplicons from these same ticks with the OmpA primers revealed two slightly different sequences, the first of which (GenBank MK882594, isolate 15SHT-071) matched with 100% identity to *I. scapularis* endosymbiont isolate TX136 (GenBank EF689737), and the second (GenBank MK882595, isolate 15SHT-031) which matched with 100% identity to *I. scapularis* endosymbiont isolate APT13-081 (GenBank KX772759) and clone FLAC1 (GenBank KX001996). Another group of gltA sequences, (GenBank MK882592, isolate 15SHT-085), had 99% identity to isolates of *Rickettsia raoultii* (GenBank MG022120) and *Rickettsia aeschlimannii* (GenBank MH064445). OmpA sequences generated from these ticks (GenBank MK882596, isolate 15SHT-085) had 100% identity to uncultured *Rickettsia* sp. clone FLAC6 (GenBank accession number KX002002). The final group of gltA sequences (GenBank MK882593, isolate 15SHT-037) had 98% identity to an unnamed *Rickettsia* sp. A12.2646 isolated from mosquitoes in Korea (accession number KY799066), and 97% identity to a *Rickettsia* sp. from fleas (accession number KY445726). These samples did not generate amplicons with the OmpA primers.

Additionally, gltA sequences matching *R. amblyommatis* were also detected in two *I. scapularis* ticks, and gltA sequences matching isolate 15SHT-037 (GenBank MK882593) were amplified from one *A. americanum* and one *D. variabilis*. While *R. amblyommatis* was detected in both nymphal and adult *A. americanum*, all of the *I. scapularis* ticks confirmed positive for *Rickettsia* spp. were adults. *Rickettsia* infection prevalence in *I. scapularis* ticks was higher in ticks collected from unburned transects compared to burned transects ($p = 0.049$). This could not be assessed for *A. americanum* due to the low sample size of *A. americanum* collected on the burned transects.

Of the 277 on-host and questing ticks tested via PCR for *Borrelia* spp. (Tables 2 and 3), 8 questing ticks generated amplicons of sufficient quality to sequence on the initial IGS PCR, but only two of those were repeatable: an *A. americanum* and an *I. scapularis* with sequences that matched to *B. lonestari*. Only one was confirmed by the qPCR: an *A. americanum* tick collected via drag sampling from the UB2 transect was positive for a relapsing fever spirochete on the qPCR and had an IGS sequence with 98% identity to *B. lonestari* (accession number AY363709). Manual inspection of the IGS chromatograph revealed at least 5 positions with double-nucleotide peaks. This same tick was also infected with *R. amblyommatis*.

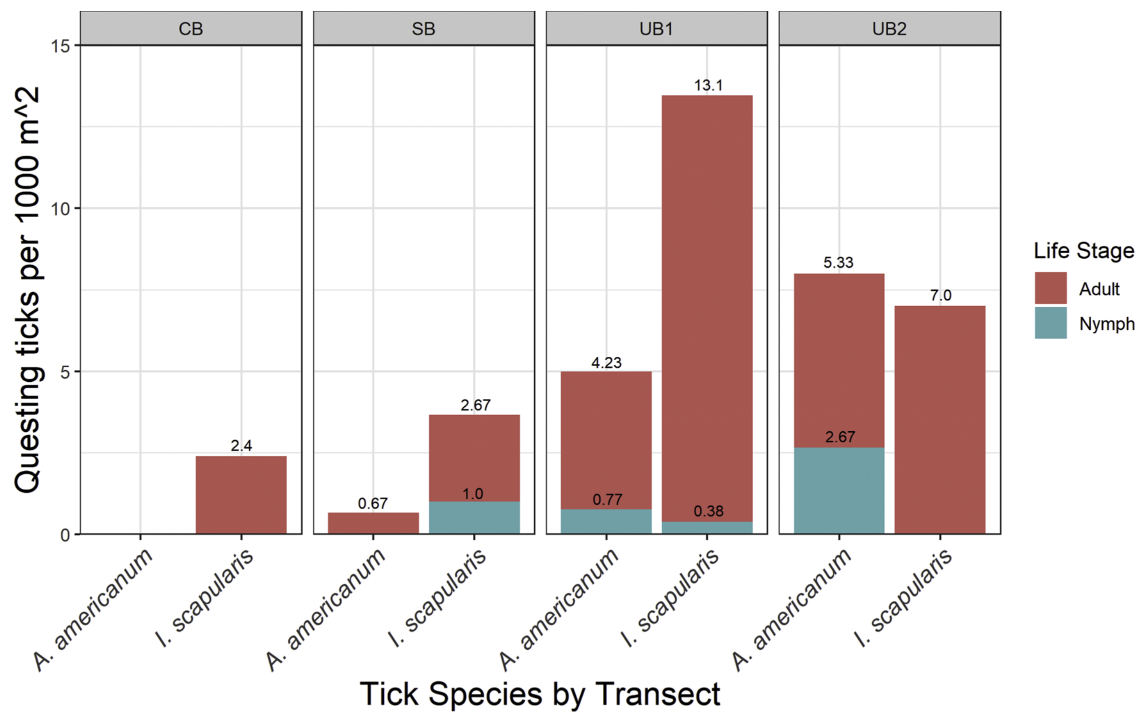

Fig. 2. Stacked bar plot displaying the density of questing ticks collected by drag sampling of four different transects at Sam Houston National Forest Stubblefield Recreation Area in east Texas in early Spring 2015. UB1 and UB2 are unburned transects, while CB and SB have been burned. The numbers above each bar represent the density (number of ticks per 1000 m^2) for that life stage.

Ear tissue from 64 and blood from 100 mammals was tested for *Rickettsia* and *Borrelia* DNA via PCR. *Rickettsia* and *Borrelia* DNA was not detected in any of the mammal ear tissue or blood samples tested.

4. Discussion

During spring sampling of a national forest recreation area in East Texas, we found a marked difference in density of host-seeking ticks between transects with different burn histories and an overall low prevalence of known pathogens. *Ixodes scapularis* and *A. americanum* were the only tick species collected by drag sampling and the adult life stage predominated for both species, although a small number of *I. scapularis* nymphs were collected by drag sampling. Other studies also report very low numbers of host-seeking *I. scapularis* nymphs collected via drag sampling in southern states (Diuk-Wasser et al., 2006; Goddard and Piesman, 2006; Ogden et al., 2018; Pepin et al., 2012; Tietjen et al., 2019). The detection of any questing *I. scapularis* nymphs in a southern state is noteworthy, as nymphal questing behavior has been correlated with geographical variation in Lyme borreliosis risk, with nymphs from high-risk northern areas more likely to quest above the leaf litter than nymphs from southern states (Arsnoe et al., 2019). Ticks infesting rodents were almost exclusively *D. variabilis* larvae or nymphs. Despite the presence of immature *D. variabilis* on rodents, this species was not collected in any life stage via drag sampling. The phenology of this species is not well characterized and adults may be active at other times of the year. Additionally, if questing activity of immature ticks was restricted to times of day or night when we did not sample, or if their occurrence was patchy in the vegetation, they could have been missed in our sampling efforts.

The average tick density on the unburned (UB) transects was approximately five times that of the average of the burned (SB and CB) transects. This supports the results of previous studies reporting reduced tick densities or encounter rates in burned areas (Gleim et al., 2014, Gleim et al., 2019; Wilson, 1986). The comparatively low tick density on the CB transect, which was burned regularly in the past but not for the last 8 years, was an unexpected finding as it is inconsistent with previous reports of tick populations rebounding quickly following cessation of burn events (Davidson et al., 1994; Stafford et al., 1998; Wilson, 1986). While vegetation density was not systematically measured in the current study, both UB compartments subjectively had much thicker undergrowth than either the SB or CB compartments. The UB2 and SB transects were both along hiking trails, and the significant difference in tick density between these two illustrates the potential for vegetation management to effectively reduce tick encounter by hikers. However, because we had a low number of transects per burn area, other unmeasured factors such as specific vegetation composition,

Table 1
Rodent captures, trap effort and success, and number and species of ticks collected from rodents trapped at Sam Houston National Forest Stubblefield Recreation Area over five visits from March to April 2015.

Transect	No. of rodent captures	No. of recaptures	Effective trap nights	Captures per trap night	No. of rodents with ticks	Total ticks on rodents	Ticks per rodent captured	Ticks per parasitized rodent	*Dermacentor variabilis*		*Ixodes scapularis*	
									Larval pools	Nymph	Larval pools	Nymph
SB	17	3	196	0.09	4	5	0.29	1.25	2	2	0	0
CB	31	8	197.5	0.16	4	4	0.13	1	1	5	0	0
UB1	43	13	194	0.22	18	66	1.53	3.67	15	25	0	2
UB2	15	2	197	0.08	4	7	0.47	1.75	2	5	0	0

Table 2
Identification and prevalence of *Rickettsia* and *Borrelia* spp. detected in questing ticks collected during drag sampling from Sam Houston National Forest Stubblefield Recreation Area. These include ticks collected from drag cloths as well as non-embedded ticks collected from humans and dogs during drag sampling efforts. *Rickettsia* and *Borrelia* spp. were examined in 32 nymphs and 186 adults.

Tick Species and Life Stage		Transect Type	No. tested for *Rickettsia*	No. infected (%) with *Rickettsia* sp.				No. tested for *Borrelia*	No. infected (%)
				R. amblyommatis	*Ixodes scapularis* endosymbiont	Uncultured *Rickettsia* 15SHT-085	Uncultured *Rickettsia* 15SHT-037		
Ixodes scapularis									
	Adult	Burned	**14**	0	6(43)	0	0	**14**	0
		Unburned	**94**	0	50(53)	10(11)	10(11)	**94**	0
		Unknown	**23**	0	5(22)	4(17)	5(22)	**23**	0
	Nymph	Burned	**3**	1(33)	0	0	0	**3**	0
		Unburned	**1**	0	0	0	0	**1**	0
		Unknown	**1**	1(100)	0	0	0	**1**	0
Amblyomma americanum									
	Adult	Burned	**2**	1(50)	0	0	0	**2**	0
		Unburned	**42**	25(60)	0	0	0	**42**	1(2) *B. lonestari*
		Unknown	**11**	9(82)	0	0	1(9)	**11**	0
	Nymph	Burned	**0**	NA	NA	NA	NA	**0**	NA
		Unburned	**21**	7(3)	0	0	0	**21**	0
		Unknown	**6**	1(17)	0	0	0	**6**	0
Total			**218**	**43(19.7)**	**68(31.2)**	**14(6.4)**	**17(7.8)**	**218**	**1(0.46)**

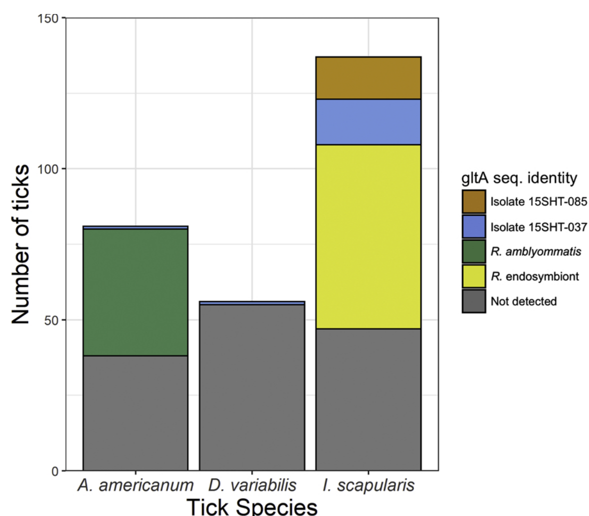

Fig. 3. Stacked bar plot displaying the results of PCR and sequencing of *Rickettsia* spp. gltA gene from three species of ticks (*Amblyomma americanum*, *Dermacentor variabilis*, *Ixodes scapularis*) collected in the Sam Houston National Forest Stubblefield Recreation Area in east Texas in early Spring 2015.

canopy cover, leaf litter depth, and microclimatic features may have played a role in tick density, and future studies should include additional transects across multiple burn sectors.

Similar to the findings of recent studies in Florida (Sayler et al., 2017) and Oklahoma (Dubie et al., 2018), we detected a number of *Rickettsia* spp. that may be endosymbionts but no known *Rickettsia* pathogens. *Rickettsia amblyommatis* was detected in *A. americanum* adults (63.6%) and nymphs (28.6%). *Rickettsia amblyommatis* is widespread in *A. americanum* ticks across the U.S. with reported prevalence ranging from 33 to 82% (Gaines et al., 2014; Jiang et al., 2010; Mixson et al., 2006; Sayler et al., 2017; Smith et al., 2010). This organism is generally considered to be an endosymbiont of *A. americanum*, but some reports suggest possible zoonotic potential (Apperson et al., 2008; Billeter et al., 2007; Hermance et al., 2014). *Rickettsia* DNA was detected in a majority (67%) of *I. scapularis* ticks, apparently representing 3 different organisms: two unnamed *Rickettsia* spp. previously reported in *I. scapularis* (Sayler et al., 2017), and a third (clade 2) whose gltA sequence matched most closely to *Rickettsia* spp. from mosquitoes and fleas and did not amplify with our OmpA primers. This sequence may represent a previously undescribed species, but sequencing additional gene regions would be needed. *Ixodes scapularis* ticks collected from unburned transects had a higher *Rickettsia* infection prevalence than those collected from burned transects; this is an interesting result which warrants further study.

Table 3
Identification and prevalence of *Rickettsia* and *Borrelia* spp. detected in ticks collected from rodents captured in Sam Houston National Forest Stubblefield Recreation Area. *Rickettsia* and *Borrelia* spp. were examined in 20 larval pools (which included 43 individual larvae) and 40 nymphs.

Tick Species and Life Stage		Transect Type	No. tested for *Rickettsia*	No. infected (%) with *Rickettsia* sp.				No. tested for *Borrelia*	No. infected (%)
				R. amblyommatis	*Ixodes scapularis* endosymbiont	Uncultured *Rickettsia* 15SHT-085	Uncultured *Rickettsia* 15SHT-037		
Ixodes scapularis									
	Nymph	Burned	**0**	NA	NA	NA	NA	**0**	NA
		Unburned	**2**	0	0	0	0	**2**	0
Dermacentor variabilis									
	Nymph	Burned	**7**	0	0	0	0	**7**	0
		Unburned	**31**	0	0	0	1(3)	**31**	0
	Larval pool	Burned	**3**	0	0	0	0	**3**	0
		Unburned	**17**	0	0	0	0	**17**	0
Total			**60**	**0**	**0**	**0**	**1(2)**	**60**	**0**

We detected *B. lonestari* in one *A. americanum* tick. This relapsing-fever group spirochete is of uncertain pathogenic significance. It was once suspected to be the causative agent of STARI (James et al., 2001). While subsequent studies have since confirmed the association between STARI and *A. americanum* (Masters et al., 2008), they have failed to provide further support for *B. lonestari* as the cause (Stromdahl and Hickling, 2012; Wormser et al., 2005).

We did not detect *B. burgdorferi* s.s. DNA in any of 278 total ticks, including 136 *I. scapularis*. This is consistent with most previous reports of low prevalence of this pathogen in ticks, animals, and humans across the southern U.S. (Bowman et al., 2009; Dubie et al., 2018; Little et al., 2014; Stromdahl and Hickling, 2012; Teltow et al., 1991; Williamson et al., 2010; Yancey et al., 2014). Specifically, studies of ticks removed from persons in Texas from 2004–2008 and 2008–2014 each detected *B. burgdorferi* s.s. in only a single *I. scapularis* of 52 and 53 tested (< 2%), respectively (Mitchell et al., 2016; Williamson et al., 2010).

We did not detect any *Rickettsia* or *Borrelia* in rodent ears or blood. Both blood and ear tissue are common and appropriate samples for tick-borne pathogen testing of reservoirs, with ear tissue being more sensitive in some cases (Schex et al., 2011; Sinsky and Piesman, 1989). However, most of the *Rickettsia* species we detected in ticks in this study are known or suspected to be endosymbionts of ticks, which likely explains their absence in the mammalian hosts.

The presence and risk of specific tick-borne diseases in the southern United States is poorly understood by the public and by many medical professionals. Results of a survey indicate that a majority of people in the south-central United States believe they are at a significant risk of contracting Lyme disease, and have very low awareness of other tick-borne diseases (Hook et al., 2015; Stromdahl and Hickling, 2012; Williamson et al., 2010). Aside from tick-borne pathogens, tick exposure still carries risks; development of a severe allergy to red meat has been associated with the bite of *A. americanum* (Commins and Platts-Mills, 2013). Additionally, other tick-borne pathogens, including viruses and protozoa, may be present in these populations but we did not test for them in this study. Furthermore, several tick-borne bacteria now recognized as potential pathogens were once thought to be harmless endosymbionts [ie: *Rickettsia parkeri* (Paddock et al., 2004), *Rickettsia monacensis* (Jado et al., 2007)] or have been shown to have evolved from endosymbionts [ie: *Coxiella burnetii* (Duron et al., 2015)], so understanding their prevalence and distribution is important.

Controlled burns may affect tick populations, pathogen prevalence, and risk of pathogen exposure to human and animals and therefore may be a useful tool in integrated tick management. The results observed here provide preliminary support suggesting fire management of forests could provide further benefits by reducing tick-borne disease risk by reducing contact with vectors, but additional work is needed to cover a larger area with multiple transect replicates and span multiple seasons. Further studies should confirm if this pattern remains true in other regions of the South receiving fire management. The current study improves the understanding of tick-borne disease ecology of mammal, tick, and pathogen communities to improve public health management.

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