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Research

Collaborative-Tick Surveillance Works: An Academic and Government Partnership for Tick Surveillance in the Southeastern United States (Acari: Ixodidae)

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Abstract

Tick surveillance provides essential information on distributions and encounter frequencies; it is a component of operational activities in public health practice. Our research objectives were a proof-of-concept for collaborative surveillance, which involved establishing an academic and government partnership to enhance tick surveillance efforts. The University of Tennessee (UT) collaborated with United States Department of Agriculture Forest Service, Southern Research Station Forest Inventory Analysis (FIA) in an Occupational Health and Safety partnership. UT provided FIA crews in the southeastern United States with vials containing 80% ethanol (July 2014–November 2017). Crew members were instructed to put all encountered ticks into the vials and return them to FIA headquarters. UT identified all submitted ticks to species and life stage, and screened *Amblyomma americanum* (L.) for *Ehrlichia* bacteria using a nested-PCR assay. From the 198 returned vials, 1,180 ticks were submitted, including *A. americanum* (90.51%; 202 larvae, 503 nymphs, and 363 adults), *Dermacentor variabilis* Say (7.12%; 1 nymph, 83 adults), *Ixodes scapularis* (Say) (1.61%; 19 adults), *Amblyomma maculatum* Koch (0.59%; 1 nymph, 6 adults), and *Amblyomma cajennense* (Fabricius) (0.17%; 1 nymph, 1 adult). FIA crews encountered *A. americanum* with *Ehrlichia* and collection information was used to generate baseline occurrence data of tick encounters. Results indicate that this collaborative-tick surveillance can be improved and used to generate useful data including pathogen detection, and because crews revisit these sites, changes in tick encounters can be monitored.

Key words: tick, passive surveillance, southeastern United States, *Amblyomma*, *Ehrlichia*

With an increase in the number and complexity of ticks and their pathogens, there is a pressing need to increase tick surveillance. The southeastern United States has a diverse and abundant tick population that is poorly understood. Specifically, current surveillance measures for ticks in the region are limited to state reports (e.g., Moncayo et al. 2010, Fritzen et al. 2011, Gaines et al. 2014, Barrett et al. 2015), opportunistic collections (e.g., gathered from tick encounters; Stromdahl et al. 2001, Lee et al. 2014), and intermittent targeted sampling (e.g., collected by university researchers; Mixson et al. 2006, Apperson et al. 2008, Scott et al. 2010, Trout et al. 2010, Nadolny et al. 2014, Wright et al. 2014, Santos and Goddard 2015, Trout Fryxell et al. 2017). Citizen-science projects provide a method for passive surveillance (Vu Hai et al. 2014, Xu et al. 2016, Nieto et al. 2018); however, these projects are challenging as nontargets are often encountered (e.g., spiders and beetles), citizens may equate pathogen-screening results with disease diagnosis, and participants may become frustrated from delayed results. Additionally, people tend to focus on Lyme disease, perhaps due to lack of awareness of prevalent regional diseases. Thus, there is a need to develop innovative collaborations for tick surveillance. Ideally, this 'collaborative-tick surveillance' will be mutually beneficial, have a goal of preventing tick encounters and subsequent tick bites and prevent infection, while simultaneously providing affordable and reliable operational activities and data for public health practices.

One of the most extensive and informative tick surveillance methods in the United States is passive tick surveillance via the Human tick test kit program of the U.S. Army Center for Health Promotion and Preventive Medicine (Stromdahl et al. 2001). Civilian and military personnel encounter ticks during field training and as ticks are encountered they are reported to medical staff on

site. Medical staff then documents each encounter with date and site information and ticks are sent to the Human Tick Test Kit Program where they are identified to species and life stage and screened for pathogens. This passive and opportunistic surveillance is mutually beneficial because it protects personnel associated with the U.S. military and provides information on tick encounters at military training sites in different states. This information has been the basis for identifying ticks and their pathogens throughout the United States and seasonal information on tick/pathogen encounters (Stromdahl et al. 2001, 2008, 2011; Loftis et al. 2008).

Many have highlighted the importance of non-*Borrelia*–related pathogens and vectors in the southeastern United States (Yabsley et al. 2005, Cohen et al. 2010, Beall et al. 2012, Stromdahl and Hickling 2012, Lee et al. 2014, Nadolny et al. 2014, Maegli et al. 2016, Kakumanu et al. 2018). When investigating human tick encounters by military personnel, Stromdahl and Hickling (2012) report *Amblyomma americanum* (L.) as the dominant tick species (>85%), followed by *Dermacentor variabilis* Say (~10%). They also reported encounters of *Ixodes scapularis* Say along the South Carolina coast, as well as Amblyomma maculatum Koch and *Rhipicephalus sanguineus* Latreille (Stromdahl and Hickling 2012). Lee et al. (2014) reported similar species encounters from North Carolina outdoor workers; outdoor workers submitted 874 ticks of which 93.8% were *A. americanum* and few were *D. variabilis*, *A. maculatum*, and *I. scapularis*.

To enhance tick surveillance efforts for the southeastern region, the University of Tennessee (UT) and United States Department of Agriculture (USDA) Forest Service, Southern Research Station Forest Inventory and Analysis (FIA) partnered in a unique academic–government collaboration. The FIA program conducts year-round, comprehensive inventories and analyses of the extent, condition, and health of forested lands across the United States. Within the Southern Research Station, FIA is responsible for 13 states, from Texas and Oklahoma east to Virginia, plus Puerto Rico and the U.S. Virgin Islands. The FIA program is unique in that many sites are scheduled for repeat visits (every 5, 7, or 10 yr) and during each visit crews inventory the site in detail for forest resources (e.g., vegetation identification and biomass) to determine annual trends in forested ecosystems (Bechtold and Patterson 2005). Additionally, crews are trained in forest safety and the importance of proper data collection. The combination of repeat visits, uniformed digital inventorying of forest resources, and field-trained crews makes this a valuable opportunity for collaborative surveillance necessary to monitor tick population changes and identification for potential reasons for those changes.

The risk for tick encounters by FIA field personnel is comparable to other agencies used for opportunistic tick surveillance (e.g., military personnel and park services). Knowing that FIA personnel have extensive biological field training and are exposed to ticks year-round, and that tick encounters are a known occupational hazard, we hypothesized that FIA crews could establish a tick-surveillance baseline against which future changes could be monitored and then compared with site-specific changes throughout the region. Our research objectives were a proof-of-concept for collaborative surveillance, which involved establishing an academic and government partnership to enhance tick surveillance efforts. Submissions were used to determine whether we could enhance tick surveillance efforts, describe seasonal and regional tick distributions from collections, and determine whether collected ticks could be screened for pathogens. We analyzed forester-provided tick collections over a 4-yr period (2014–2017) and used those data to generate baseline presence maps and seasonality of ticks in the southeast. We also screened the most commonly encountered species—*A. americanum*—for *Ehrlichia* bacteria to determine whether these collections could be used for pathogen surveillance.

Methods

Site Description

FIA sites consist of fixed sites on all land ownerships, one site per every 5,937.2 acres. Each site is located randomly within its 5,937.2-acre hexagonal cell, so sites may be currently or previously forested, or may be any land use (e.g., residence, agriculture, and urban forest). The sample is stratified via photointerpretation of remotely sensed data such that forested sites are sampled on the ground with relatively few nonforested or difficult to interpret sites included as a form of quality control. The FIA experimental design and sampling protocol (Bechtold and Patterson 2005) are designed to capture boundaries between various conditions such as forest type, within each FIA site four discrete plots are noted and drawn during sampling. Each year a proportion (1/5, 1/7, or 1/10 varying by state and crew availability) of the total sites in each state are sampled—this is referred to as a panel. Sites within a panel are spatially distributed throughout the state, and FIA provides an annual update of the estimated forest resources for each state. For a full description of the FIA sampling design, see Bechtold and Patterson (2005). Consequently, our 4-yr collaboration had the potential to include approximately 74 crews visiting 45,244 sites within the southeastern states which included Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, Texas, Virginia, and West Virginia.

Tick Collection and Identification

FIA foresters voluntarily participated in opportunistic collections of ticks encountered during their fieldwork. After giving a tick-related field-safety talk to crews in the spring of the initial year, all crews were provided collection kits with instructions on tick removal, vials with 80% ethanol, and magnifying forceps. Crews were requested to collect and store any encountered tick (defined as a tick that attached or found unattached onto their person) into the vial; an asterisk was to be placed on vials that had attached ticks. Each vial was then labeled with their crew number, date of encounter, and site the crew visited. Crew members had ample opportunity (a minimum of 10 min to a maximum of 6 h per site) to encounter ticks, while navigating to or working at field sites. Nonforest sites take 10 min to 1 h for the crew to determine tree cover (<10%, nonforest), whereas a forested site would take 1–6 h depending on location, stocking, and complexity. Time in a site is not standardized and we do not know how often personnel checked themselves for ticks and/or if all ticks encountered were collected and submitted. The minimum distance traveled to navigate to and from each subsite from site center, and walk the circumference of each subsite is about 400 m (1300 ft). We recognize that if two sites were visited by the same crew on the same day, encountered ticks may have come from either site; however, USDA–FIA encouraged personnel to inspect themselves upon completing each site to minimize the chance of having a tick attach and misreporting an encountered tick. Opportunistically collected ticks were then submitted to USDA–FIA headquarters and then to the UT Medical and Veterinary Entomology laboratory. At the laboratory, ticks were identified to species, sex, and life stage using dichotomous keys (Cooley and Kohls 1945, Clifford et al. 1961, Keirans and Litwak 1989, Durden and Keirans 1996, Keirans and Durden 1998, Nava et al. 2014).

Resulting submission data were used to create presence maps using ArcGIS 10.3.1 (ESRI, Redlands, CA). To introduce uncertainty into the data with regard to land ownership, satisfying legal requirements, and privacy concerns, as well as to protect the integrity of the sites, our reported site data are not exact. Coordinates are fuzzed (when the site location is slightly altered and relocated within 1 mile of the actual site) and sites occurring on private lands are swapped (some site coordinates are randomly swapped with ecologically similar sites based on forest type group and stand size within a county). For the purposes of our reporting herein the effect of fuzzing and swapping is negligible, it is a mechanism to protect site locations.

We also calculated descriptive statistics such as mean, standard deviation, total, range, 90% confidence intervals, prevalence (percentage of crews within sampled group that were infested with ticks), and tick burden (mean number of ticks found on infested crews) for each state and tick species and life stage encountered.

Ehrlichia Detection

Ticks submitted from 2014 to 2016 identified as *A. americanum* were then screened for *Ehrlichia* DNA using previously described procedures. Briefly, the DNA of individual *A. americanum* ticks were extracted with the Fermentas Gene Jet Genomic DNA purification kit and protocol yielding 200 µl of DNA eluted in buffer (ThermoFisher Scientific, Pittsburgh, PA). Three microliters of eluted DNA was then subjected to *groEL* amplification via a nested-PCR assay using Maxima Hot Start Green PCR Master Mix (Thermo Scientific, Pittsburgh, PA) to determine the presence of *Anaplasma/Ehrlichia* DNA (Tabara et al. 2007, Takano et al. 2009, Trout Fryxell et al. 2017). One positive control (previous *Ehrlichia*-positive tick) and three negative controls (water, MasterMix without DNA, and previous *Ehrlichia*-negative tick) were used. If a tick was positive (presence of band in a 1.5% agarose gel: 1 X TAE buffer with ethidium bromide for 2 h at 120 V), then that amplicon was bidirectionally sequenced at the UT sequencing facility using Sanger sequencing and resulting sequences were compared with GenBank deposits as described previously (Trout Fryxell et al. 2017).

Results

Crew Participation

Over the entire period (July 2014–November 2017), a total of 30 (40%) unique FIA crews participated in the collection and submitted 226 vials. Data were complete for 198 vials (87.6% correctly labeled and returned). The 28 discarded vials were not used in analyses because they did not contain complete collection information (e.g., date, site, crew identification, and/or GPS coordinates); unfortunately, this included 159 tick specimens representing *A. americanum, D. variabilis,* and *I. scapularis*. Two of the 198 vials had no ticks; vials were returned empty but collection data were properly recorded. This gave us a total of 196 vials with specimens and complete labels for analysis.

FIA crews encountered ticks throughout the year, but more crews encountered ticks from April through September as indicated by vial submissions (Fig. 1A); ticks during that period were primarily *A. americanum* and *D. variabilis*. Outside of what a FIA crew

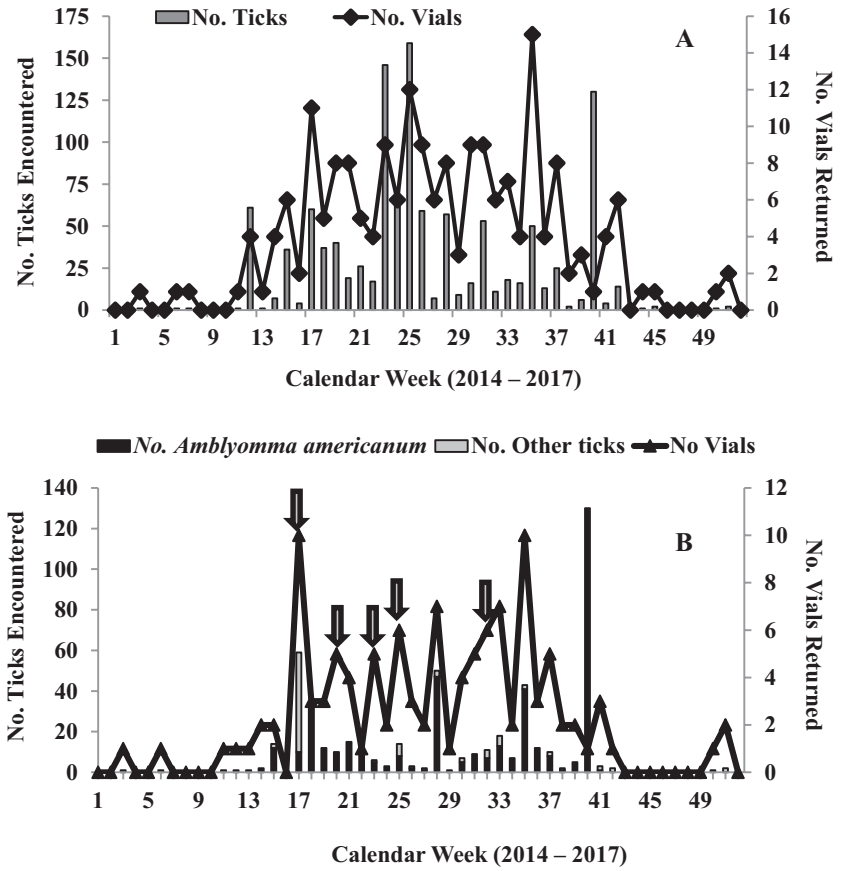

Fig. 1. FIA personnel encountered ticks and returned them in vials from July 2014 through November 2017 (A). From 2014 through 2016, *A. americanum* were screened for *Ehrlichia,* arrows indicate calendar week when a positive tick was encountered (B).

Table 1. USDA–FIA forest service submitted vials with ticks to the University of Tennessee

Tick encounters	Southeastern state (no. of potential crews)													
	AL (6)	AR (5)	FL (6)	GA (6)	KY (5)	LA (7)	MS (6)	NC (5)	OK (3)	SC (4)	TN (5)	TX (12)	VA (4)	Total (74)
							2014							
No. of crews	0	1	2	0	0	0	0	1	1	0	0	1	1	7
No. of vials	0	4	8	0	0	0	0	1	6	0	0	1	1	21
No. of ticks	0	4	12	0	0	0	0	1	16	0	0	2	2	37
Mean (SD)		1 (0)	1.10 (1.100)						2.67 (1.211)					1.76 (1.091)
Range			1–4						1–4					
90% CI		1	0.46– 1.74						1.85–3.48					1.37– 2.15
Prevalence		0.20	0.33					0.2	0.33				0.25	0.09
Burden		1	0.55						2.67					0.25
							2015							
No. of cews	0	1	2	0	0	0	1	1			5			10
No. of vials	0	6	10	0	0	0	1	2			23			42
No. of ticks	0	9	45	0	0	0	1	8			84			147
Mean (SD)		1.50 (0.548)	4.50 (10.721)					4.00 (1.141)			3.65 (6.407)			3.50 (6.954)
Range		1–2	1–35					3–5			1–30			1–35
90% CI		1.13–1.87	–1.08–10.08					2.67–5.33			1.45– 5.85			1.74– 5.26
Prevalence		0.2	0.33					0.2			1			0.14
Burden		1.5	2.25					4			0.73			0.35
							2016							
No. of crews	0	1	1	0	2	1	0	0	0	0	3	0	1	9
No. of vials	0	5	1	0	24	1	0	0	0	0	18	0	3	52
No. of ticks	0	5	1	0	216	1	0	0	0	0	92	0	5	320
Mean (SD)					9.00 (26.113)						5.11 (7.136)		1.67 (1.155)	6.15 (18.264)
Range					0–130						1–29		1–3	0–130
90% CI					0.23–17.77						2.34–7.88		0.57–2.76	1.99–10.32
Prevalence					0.4						0.6		0.25	0.12
Burden					4.5						1.70		1.67	0.68
							2017							
No. of crews	1	1	2	0	8	1	0	0	1	3	2	0	0	19
No. of vials	1	2	10	0	50	2	0	0	2	9	7	0	0	83
No. of ticks	53	3	42	0	402	3	0	0	2	10	161	0	0	676
Mean (SD)		1.50 (0.707)	4.20 (5.266)		8.04 (14.371)	1.50 (0.707)				1.11 (0.333)	23.00 (38.566)			8.14 (17.012)
Range		1–2	1–18		1–74	1–2				1–2	1–106			1–106
90% CI		0.68–2.32	1.46–6.94		4.70–11.38	0.68–2.32				0.93–1.29	–0.98–46.98			5.07–11.22
Prevalence		0.20	0.33		1.60	0.14				0.75	0.40			0.26
Burden		1.50	2.10		1.01	1.50				0.37	11.50			0.43

Table 1. Continued

Tick encounters	Southeastern state (no. of potential crews)													
	AL (6)	AR (5)	FL (6)	GA (6)	KY (5)	LA (7)	MS (6)	NC (5)	OK (3)	SC (4)	TN (5)	TX (12)	VA (4)	Total (74)
	Overall													
No. of crews	1	1	3	0	8	1	1	1	2	3	6	1	2	30
No. of vials	1	17	29	0	74	3	1	3	8	9	48	1	4	198
No. of ticks	53	21	100	0	618	4	1	9	18	10	337	2	7	1180
Mean (SD)		1.24 (0.437)	3.45 (6.931)		8.35 (18.806)	1.33 (0.577)		3.00 (2.000)	2.25 (1.282)	1.11 (0.333)	7.02 (16.507)		1.75 (0.957)	5.96 (14.906)
Range		1–2	1–35		0–130	1–2		1–5	1–4	1–2	1–106		1–3	0–130
90% CI		1.06–1.41	1.33–5.57		4.76–11.95	0.79–1.88		1.10–4.90	1.50–3.00	0.93–1.29	3.10–10.94		0.96–2.54	4.22–7.70
Prevalence		0.20	0.50		1.60	0.14		0.20	0.67	0.75	1.20		0.50	0.41
Burden		1.24	1.15		1.04	1.33		3.00	1.13	0.37	1.17		0.88	0.20

Summary statistics including count data (crews participating, vials submitted, and ticks submitted), mean number of ticks per vial, range of ticks per vial, 90% confidence interval, prevalence (percentage of crews within sampled group that were infested with ticks), and tick burden (mean number of ticks found on infested crews) were determined for each state and year.

may describe as 'tick season' (November–February), the primary tick encountered was *I. scapularis*. We started with six states and seven crews voluntarily participating in 2014 and quickly grew to eight states and 19 crews participating in 2017 (Table 1). Tick collections reflected this increase from 42 ticks representing four tick species in 2014 to 1004 ticks, representing four tick species collected in 2017. In total, crews from 12 of the 14 southeastern states submitted at least one vial.

Tick Encounters

In total, 1,180 ticks were encountered and properly documented by USDA–FIA crews over the 4-yr window (Fig. 1A). Samples ranged from 0 to 130 ticks per vial with a mean (SD) of 5.96 (14.906) specimens per vial. Five tick species were submitted by FIA crews: *A. americanum* (90.51%; 202 larvae, 503 nymphs, and 363 adults), *D. variabilis* (7.12%; 1 nymph, 83 adults), *I. scapularis* (1.61%; 19 adults), *A. maculatum* (0.59%; 1 nymph, 6 adults), and *Amblyomma cajennense* (Fabricius) (0.17%; 1 nymph, 1 adult; Table 2). Submissions were used to calculate descriptive statistics and encounter frequencies (number submitted that period/total number submitted) for each *A. americanum* life stage, *D. variabilis* adults, and *I. scapularis* adults (Fig. 2).

During the collection period, *A. americanum* were encountered March through October with a majority collected in the early spring months of April and May. Larvae were bimodal and were encountered in July and then again in October. Nymphs were encountered year-round, but primarily in April, June, and August. Adult populations began questing in March, peaked in June, and then rebounded in August. From March through September, *D. variabilis* were encountered and peaked in June (30.95%). Crews primarily encountered adults and these were bimodal with collections in March and again in June; the one nymph was collected in June from Kentucky. Although infrequent, one *A. maculatum* was encountered in June (28.57%), and then August through October (August, 14.29%, September, 14.29%, and October, 42.86%). These collections were from Kentucky, South Carolina, and Florida. Adult *I. scapularis* were encountered throughout the year, but primarily in the fall and winter months from October through May, peaking in November and December. Two ticks encountered in October 2014 from Jim Hogg County, TX were morphologically identified as *A. cajennense*; however, molecular identification would verify this identification as it is a part of a species complex (Nava et al. 2014).

Prevalence and tick burdens were calculated for each state, year, and overall collection (Table 1). These statistics were not calculated for the states of Alabama, Georgia, Mississippi, or Texas due to the few ticks and/or vials returned. Overall encounter prevalence was 0.41 and ranged from 0.14 (Louisiana) to 1.60 (Kentucky) indicating crews are encountering ticks because nearly half of them submitted a tick-filled vial. There was also a trend for increasing prevalence of encounters for each sampling year: 0.09 in 2014, 0.14 in 2015, 0.12 in 2016, and 0.26 in 2017. Tick burden varied by state and year, but the overall tick burden was 0.20. South Carolina had the lowest burden (0.37), whereas Tennessee had the highest (11.5), indicating Tennessee crews are encountering and collecting more ticks.

Collected data were used to create FIA crew tick-encounter maps based on our presence only data for the three *A. americanum* life stages and additional tick species (Fig. 3). *Amblyomma americanum* and *D. variabilis* were encountered throughout the sampling area. Few *A. maculatum* specimens were collected, but they were encountered in three of the participating states including Kentucky, and *I. scapularis* was encountered in four of the participating states.

Table 2. Descriptive statistics for each tick species and life stage encountered by FIA personnel

Collection information	*Amblyomma americanum*			*Amblyomma aajemense*		*Amblyomma maculatum*		*Dermacentor variabilis*		*Ixodes scapularis*	Total
Tick life stage	Larvae	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults	Adults	
No. of crews	4	14	21	1	1	1	4	1	12	7	30
No. of vials	10	101	89	1	1	1	5	1	52	16	196
No. of ticks	202	503	363	1	1	1	6	1	83	19	1180
Mean	1.02	2.54	1.83	0.01	0.01	0.01	0.03	0.01	0.42	0.10	5.96
(SD)	(9.525)	(6.761)	(7.377)	(0.071)	(0.071)	(0.071)	(1.990)	(0.071)	(1.345)	(0.343)	(14.906)
Range	0–129	0–64	0–96	0–1	0–1	0–1	0–2	0–1	0–17	0–2	0–130
90% Confidence interval	8.41–10.64	5.97–7.55	6.51–8.24	0.06–0.08	0.06–0.08	0.06–0.08	0.18–0.22	0.06–0.08	1.19–1.50	0.30–0.38	13.16–16.65
Prevalence	0.05	0.19	0.28	0.01	0.01	0.01	0.05	0.01	0.16	0.09	0.38
Burden	0.26	0.18	0.09	0.01	0.01	0.01	0.01	0.01	0.04	0.01	0.21

Prevalence was calculated as the percentage of crews within a sampled group (state, year, and overall) that were infested with ticks and tick burden was calculated as the mean number of ticks found on infested crews.

The dominant tick species (*A. americanum*, *D. variabilis*, and *I. scapularis*) were also identified at different latitudes with all three encountered between 37°N and 29°N.

Co-occurrences

Most encounters involved one tick species (86.36%, 171/198) and included 123 vials with *A. americanum*, 29 vials with *D. variabilis*, 14 vials with *I. scapularis*, 4 vials with *A. maculatum*, and 1 vial with *A. cajennense*. Of the 25 vials with co-occurrences (12.63%), this occurred 22 times with *A. americanum* and *D. variabilis*, twice with *A. americanum* and *A. maculatum*, and once with *A. americanum* and *I. scapularis*. One encounter from March 2017 in Alabama involved three tick species and contained 35 *A. americanum*, 17 *D. variabilis*, and 1 *I. scapularis*.

Ehrlichia Detection

Ehrlichia was identified from 6 (1.99%) of 301 screened *A. americanum* collected between 2014 and 2016. Two larvae (6.06%) and four adults (4.49%) were positive; none of the 179 nymphs screened were positive in the nested-PCR assay. Five of the *groEL* amplicons were 99% similar to *E. chaffeensis* (GenBank KJ907746–KJ90774753) and one *groEL* amplicon from a female *A. americanum* encountered near Halifax, North Carolina was 99% similar to *E. ewingii* (GenBank JK907744 and AF195273). The two larvae were engorged slightly and the adults were not engorged. Five different crews (of the 30 crews that submitted ticks) encountered *Ehrlichia*-infected ticks from April through August (Fig. 1B). These crews were working near Ocala (Florida), Halifax (North Carolina), Big Island (Virginia), Rockwood (Tennessee), and Rhodelia (Kentucky). Interestingly, the crew near Ocala Florida encountered and submitted *Ehrlichia*-positive larvae in subsequent years.

Discussion

Active surveillance for ticks and their pathogens is an effective means for identifying where pathogen-positive ticks are questing; however, our research aligns with others documenting that passive surveillance can be informative for large-scale tick and pathogen surveillance (Stromdahl et al. 2001, Lee et al. 2014, Barrett et al. 2015, Xu et al. 2016, Nieto et al. 2018). Our study documented that FIA crews in the southeastern region encountered and collected five different tick species, of which, *A. americanum*, *D. variabilis*, and *I. scapularis* were the most frequently submitted. Based on submission data generated from this study, we created encounter frequency graphs and tick-encounter maps for each of the five tick species and the different life stages of *A. americanum*. Our results are comparable to previous encounter and submission studies (Stromdahl and Hickling 2012, Lee et al. 2014, Nieto et al. 2018), frequent encounters of *A. americanum* and *D. variabilis*, and limited encounters of *A. cajennense*, *A. maculatum*, and *I. scapularis* throughout the region. Our limited *A. maculatum* collections align with few military personnel encounters from the southeastern United States from 2000 to 2009 which only included 35 *A. maculatum* (Jiang et al. 2012). Additionally, the phenology of our 19 *I. scapularis* aligns with recently generated models for Tennessee, South Carolina, and Florida (Ogden et al. 2018).

FIA crews properly stored specimens in ethanol-filled vials indicating we can screen their submissions for potential tick-borne pathogens. Of the 30 crews, five were exposed to *Ehrlichia*-positive *A. americanum* (16.7% of FIA crews) and these *Ehrlichia*-infected ticks represented ~2% of the screened ticks. This low *Ehrlichia*

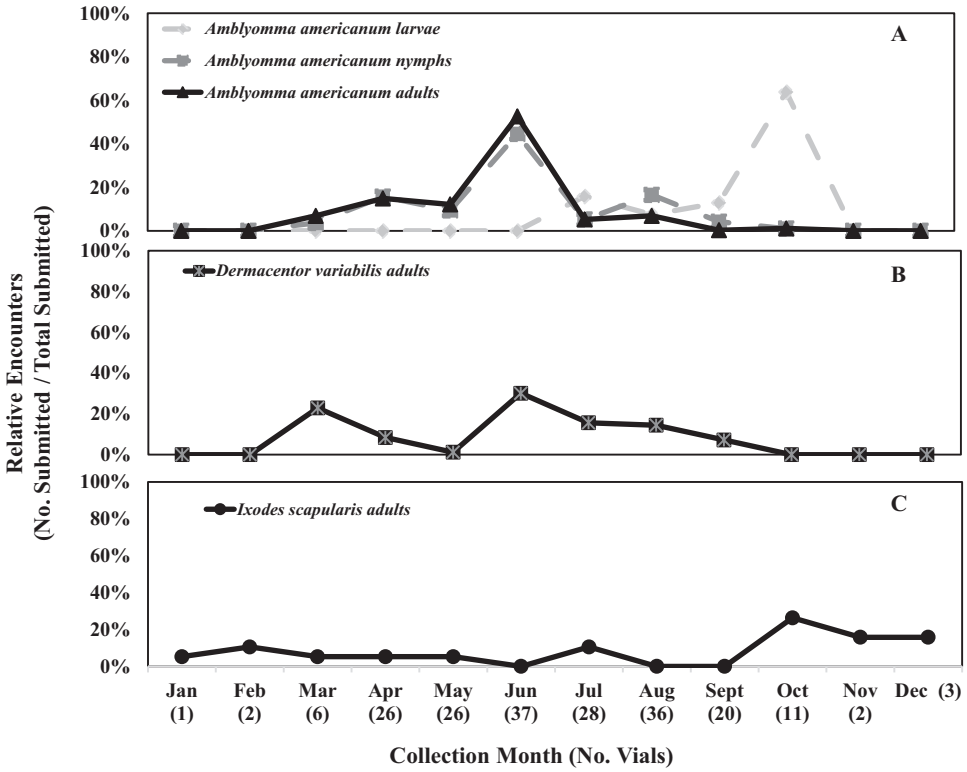

Fig. 2. Cumulative tick submissions by USDA–FIA were used to calculated encounter frequency (number submitted that period/total number submitted) for each *A. americanum* life stage (A), *D. variabilis* (B), and *I. scapularis* (C). Note that the effort is not equal across all data points and could seriously bias the results, which is related to the number of submitted vials in parentheses next to the month.

prevalence in the sampled population corroborates other surveillance studies which indicate that *Ehrlichia* prevalence in the tick population is typically less than 5% (Loftis et al. 2008, Fritzen et al. 2011, Harmon et al. 2015, Trout Fryxell et al. 2017). At neighboring sites, one crew encountered *Ehrlichia*-positive larvae in subsequent years indicating some crews may be at more risk than others. We suspect the two slightly engorged larvae started to feed on an infected host and were dislodged from that host before questing onto the FIA personnel. These data allow us to let those crews know of the risk they have when entering the field and the need for use of additional precautions as ticks were encountered year-round. As previously noted, passive surveillance can assist with determining chance of encountering ticks, infection status of the ticks, and duration of the tick bites (Xu et al. 2016). We suspect that FIA crews who participate regularly in the study are developing habits that involve checking themselves for ticks regularly, thereby, limiting the opportunity for ticks to bite and decreasing their risk for acquiring a tick-borne pathogen. To know this with certainty, follow-up studies are needed.

Our data and crew participation were skewed to the Kentucky and Tennessee area. This is likely due to the proximity of the UT and the connections between different FIA crew leaders, participation in the opportunistic collection varied throughout the region. Recruiting states to participate and retention of states participating has been an ongoing difficulty. All crews were asked to participate in the study and were solicited equally (e.g., emails, phone conversations, and provided tick-collection packets). We assume that <50% participation and retention has to do with in-place habits, work-related responsibilities, and no reward for participating in this study. We believe that participating crews are experiencing more ticks than they are used to experiencing and/or are concerned about ticks, but this can only be assessed with more tick submissions and social and human dimension studies. We consider variation in response rate to be part of our results and are considering ways to increase crew participation in underrepresented areas and enhance crew retention as studies continue. We know this initial work will help communicate the need for better coverage and will help us persuade crews in those areas to assist in the effort.

We created a unique academic–government partnership that mutually benefits both institutions. Having FIA crews check themselves for ticks has the potential to reduce the number of tick bites and subsequent infections with a pathogen (work-related illness); likewise, the data set generated from those collections can be used to generate entomological and epidemiological data such as encounter frequencies, distributions, and locations where pathogen-infected ticks are questing. Moreover, the findings that prevalence and burden varied by year and location can likely be explained by a combination of human behaviors, habitats, crew participation, and factors yet to be identified. With continued collaborations, we expect to identify unique environmental variables and generate accurate environmental and phenological models associated with each tick species. With time, we expect more crews will participate in the study. Often participation and retention will vary with citizen science and collaborative science projects (Conrad and Hilchey 2011, Rotman et al. 2012), to limit this from occurring we plan to present findings and tick-safety recommendations at semiannual SRS FIA field-staff safety engagements, Annual State Coordinator's meetings, acknowledge contributions from each state, and ask for constructive feedback. Additional benefits from this collaboration include relatively inexpensive surveillance efforts that supplement survey

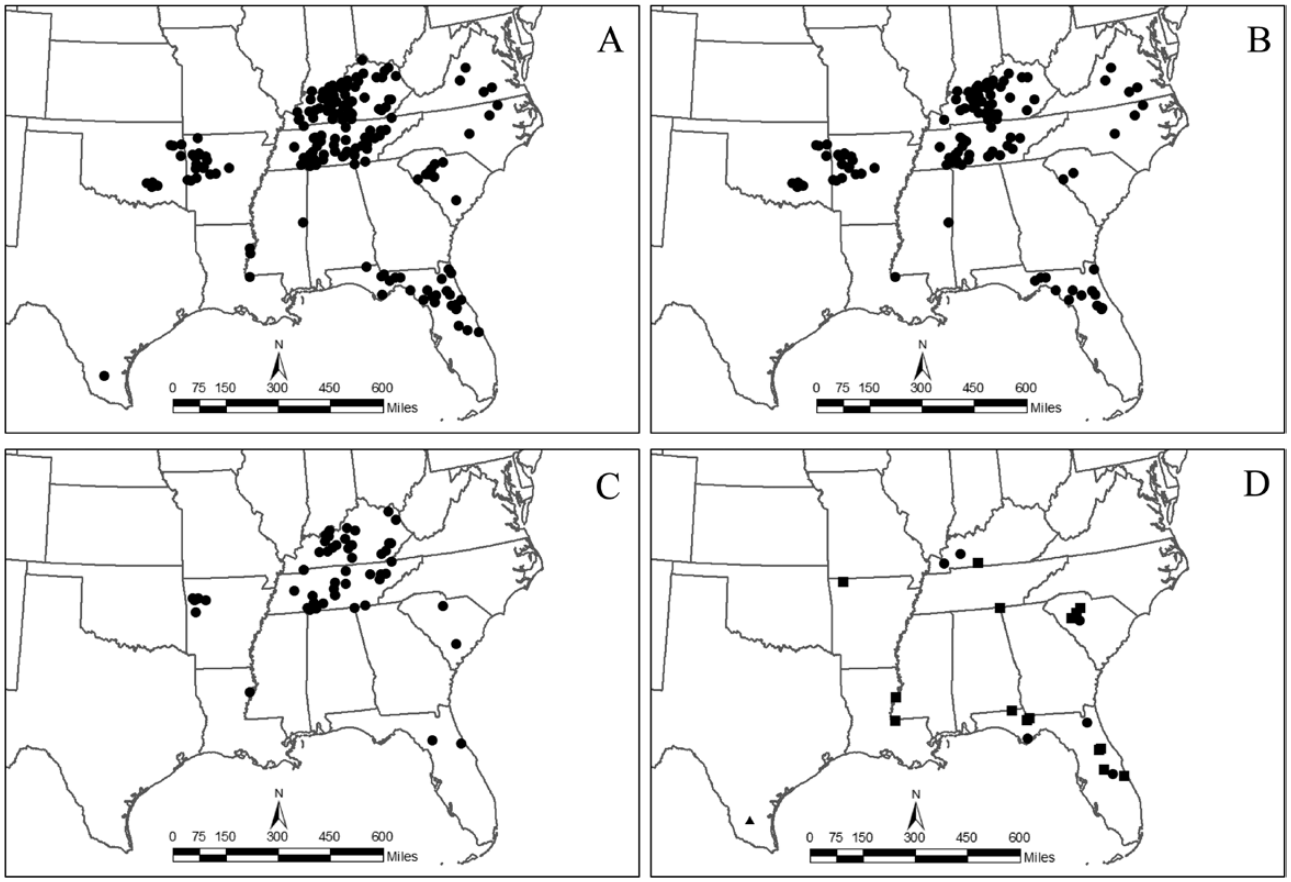

Fig. 3. FIA-encounter data of different tick species and life stages were used to generate presence maps. Sites where ticks were encountered (A). Life-stage data were combined for *A. americanum* (B) and *D. variabilis* (C). Map of encounters with rarer specimens including *A. maculatum, A. cajennense,* and *I. scapularis* (D). Graduated symbols are used to indicate abundance.

information collected by local and state agencies, provide information with benefits to public health, and provide alerts of changes through time in tick numbers or infection, which could then be further investigated. Ultimately, we are optimistic that this continued collaborative project will 1) lead to a dynamic tick (and with funding pathogen) database that will continue to expand our knowledge of tick and pathogen occurrence data regarding phenology, distribution, and encounter rates, 2) provide an increased sense of ownership among field personnel with regard to safety related to ticks and tick-borne illnesses, 3) generate an overall increased awareness of tick-borne disease and steps that can be taken to mitigate risk of bites, and 4) assumed reduced occupational risk to tick-borne diseases with increased education.

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