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Research Article

Sampling, Distribution, Dispersal

Beefing Up Biosecurity: Survey of Ticks (Acari: Ixodidae) Currently Threatening the Tennessee Beef Cattle Industry, and a Proposed Monitoring Strategy for Invasive Ticks

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Abstract

Tick-borne diseases are poised to devastate the North American cattle industry if infected ticks invade the country either by importation of an infested-animal or with natural host migration. Our research objectives were to identify sources for invasive-tick monitoring and use those sources to describe seasonal and regional impacts on infestation prevalence and burden of ticks on beef cattle. Throughout the state of Tennessee, we sampled 25% of the total herd size (or 10 animals) at three university-operated research and education centers (RECs) (sentinel sites), six livestock auctions (check-stations), and nine Extension agents at 21 producer locations (tick scouts) from 2015 to 2016. From 1,817 sampled cattle 740 ticks (Acari: Ixodidae) were collected including 573 *Amblyomma americanum* (L.) (77.4%), 125 *A. maculatum* Koch (16.9%), 35 *Dermacentor variabilis* (Say) (4.7%), and 3 *Ixodes scapularis* (Say) (0.4%). Western and middle Tennessee were significantly different in infestation prevalence and burden of *A. maculatum*. For *A. maculatum* and the species total, infestation prevalence and burden were greater in spring than fall. Auctions (check stations) and RECs (sentinels) had the greatest infestation prevalence of *A. maculatum*, and the greatest burden of *A. maculatum* and *D. variabilis*. High-risk locations clustered in western and middle Tennessee, with low-risk locations in middle and eastern Tennessee. Results from this study provide knowledge necessary to initiate control measures, including seasonal phenology and regional distribution of current tick threats. Use of RECs as sentinel sites and routine tick surveillance at livestock auctions serving as check stations should be used for mitigating invasive tick threats.

Key words: Acari, tick, cattle, surveillance, invasive biology

Ticks (Order: Acari) are blood-feeding arthropods of both medical and veterinary significance because they can damage a host via multiple mechanisms. Tick attachment can cause direct damage through dermatitis, allergies, introduction of toxic salivary compounds, and provide entry points for secondary infections (Jongejan and Uilenberg 2004). Additionally, ticks can indirectly damage their host via the transmission of pathogenic microbes. Ticks and tick-borne diseases are a serious threat to the cattle industry in the United States. The U.S. beef cattle industry significantly contributes to the country's economy, with a retail value estimated at US$105 billion in 2015 (USDA-ERS 2017). In 2012, cow-calf production for beef was one of Tennessee's top agricultural commodities at US$735.5 million (Vilsack and Clark 2014). The cattle industry's economic success is dependent upon proper management of factors that affect cattle production. Cattle health is of major importance, with annual losses from health-related issues estimated at US$20–25 million in Tennessee (Neel 2013). Although ticks likely contribute to health losses in Tennessee, anecdotal evidence indicates that many producers are unconcerned or unaware of the consequences these pests can have on cattle health. This pervasive mindset makes the cattle industry vulnerable to endemic ticks and pathogens, including those that transmit bovine anaplasmosis (BA). BA is a serious disease of cattle that occurs in many parts of the United States (Merriman et al. 1966, McCallon 1973, Whitlock et al. 2014). The etiological agent, *Anaplasma marginale* Theiler (Rickettsiales: Anaplasmataceae), can be transmitted mechanically by biting arthropods or fomites contaminated by blood, and biologically by *Dermacentor* ticks (Dikmans 1950, Kocan et al. 2004). Infected cattle can suffer from fever, anorexia, and abortions (Ristic 1977), which can negatively affect the livelihood of cattle producers. For California beef cattle, the estimated cost of direct losses from BA infection combined with treatment and control costs is US$1.48 million (Goodger et al. 1979).

Furthermore, underestimating the impact of ticks on cattle health could create conditions that allow for the invasion of new threats.

The August 2017 discovery of *Haemaphysalis longicornis* Neumann (Acari: Ixodidae) on a sheep from Hunterdon County New Jersey (Rainey et al. 2018) has serious implications for cattle health, as it is a vector of *Babesia ovata* (Piroplasmida: Babesiidae) and *Theileria orientalis* (Piroplasmida: Theileriidae) (Fujinaga 1981, Sivakumar et al. 2012). The following May, *H. longicornis* was recovered at a cow-calf operation in Virginia; ~500 km (~300 miles) from the original infestation site (Anonymous 2018). As of August 1, 2018, seven U.S. states confirmed the presence of *H. longicornis* on a variety of livestock and wildlife (unpublished). Two additional tick and pathogen complexes are primed to invade the U.S. and pose a significant risk to the cattle industry. In Mexico, *Rhipicephalus microplus* (Canestrini) (Acari: Ixodidae) and *Rhipicephalus annulatus* (Say) (Acari: Ixodidae) are vectors of *Babesia bigemina* (Piroplasmida: Babesiidae) and *Babesia bovis* (Piroplasmida: Babesiidae), the protozoans causing Bovine Babesiosis (BB). Following an eradication program to eliminate BB from the United States, efforts to prevent reestablishment of the tick vectors have consisted primarily of strict regulation and treatment of animals imported from Mexico. Resistance of *R. microplus* to acaricides used to treat cattle moving across the border (Li et al. 2003, 2004; Miller et al. 2005) and unregulated movement of suitable alternate wildlife hosts such as white-tailed deer (*Odocoileus virginianus* (Zimmermann) (Artiodactyla: Cervidae)) (Busch et al. 2014) have made breaks in the quarantined zone a grim reality. In the Caribbean, the Tropical Bont Tick (*Amblyomma variegatum* (Fabricius) (Acari: Ixodidae)) is a vector of *Ehrlichia ruminantium* Dumler (Rickettsiales: Rickettsiaceae), the bacteria causing Heartwater (HW). The invasion of this tick is possible via imported pets and livestock (Deem 1998) and/or migration of cattle egrets (*Bulbulcus ibis* (L.) (Pelecaniformes: Ardeidae)) which serve as suitable hosts for immature bont ticks (Burridge et al. 1992). While BB and HW are not currently found in the United States, they are of concern to the cattle industry because of the high estimated death loss (≥70%) (Wagner et al. 2002) and potential economic impact (Dietrich and Adams 2000) following introduction.

To prepare for these impending threats, it is vital that the cattle industry collect key information on the biology and activity of current tick threats to mitigate economic losses. Additionally, investigating surveillance approaches will foster the development of improved detection methods to prevent establishment and spread of invasive ticks. To protect the cattle industry from ticks, we are testing the overarching hypotheses that the infestation prevalence and burden of ticks will vary by season, region and collection source and that specific sources of ticks may serve as a tool for surveillance. To test this hypothesis, our objectives were to characterize the tick infestation prevalence and burdens to cattle and determine the best strategies for monitoring for invasive ticks using Tennessee cattle as the model.

Materials and Methods

Collection Sources

We used three collection sources to sample from a geographically and genetically diverse set of cattle, to collect a variety of ticks, to capture an accurate representation of tick species on cattle, and to compare the different sources for developing surveillance programs. Sites included three university-operated research and education centers (RECs) which served as sentinel sites, six livestock auctions served as check-stations, and nine Extension agents served as tick scouts and collected ticks from 21 producer locations (Fig. 1). Additionally, 12 United States Department of Agriculture (USDA) approved livestock slaughterhouses were contacted, of which zero were able to participate in this study. Before sampling, we obtained approval to collect ticks from the cattle sources via signed documentation and from the University of Tennessee (UT) Institutional Animal Care and Use Committee (IACUC) committee (IACUC #2192).

The UT RECs carry out field studies for the benefit of producers in the agricultural and natural resource industries. The RECs sites in this study were used previously in a project investigating tick-cattle associations in Tennessee where ticks were collected primarily in the western region of the state (Pompo et al. 2016). Ames Plantation (~7,446 hectares) has approximately 200 head of Angus beef cattle and is located in western Tennessee (35.114394, −89.211781) within the Mississippi Valley Loess Plains ecoregion (Griffith et al. 1998). The Middle Tennessee Research and Education Center (~511 hectares) has approximately 140 cows consisting of angus, charlois and black baldy [hereford × angus]) and is located in central Tennessee (35.718806, −86.965131) within the Interior Plateau ecoregion (Griffith et al. 1998). The Plateau Research and Education Center (~850 hectares) has approximately 200 head of Angus beef cattle and is located in eastern Tennessee (36.105349, −85.132090) within the Southwestern Appalachians ecoregion (Griffith et al. 1998). Use of each REC as a sentinel site is ideal because of the location, documented tick history, ability for routine tick surveillance, consistent

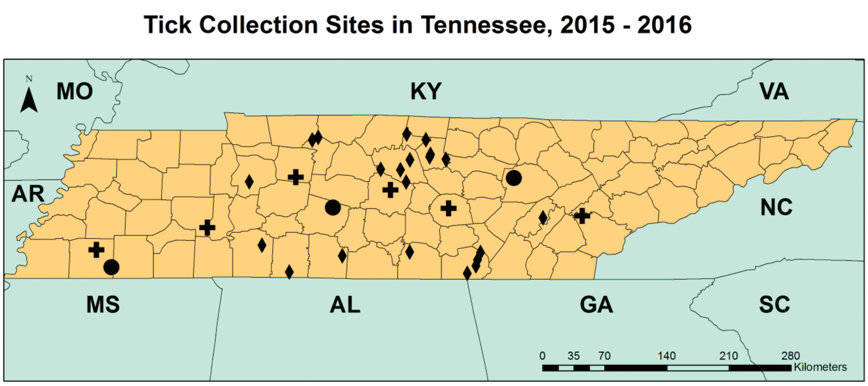

Fig. 1. Collection sources across Tennessee 2015–2016. Surrounding states are denoted by their respective U.S. Postal Service two letter code.

management practices, and abilities to document and monitor trends and control measures.

UT employs approximately 400 extension agents working in 95 offices located in every county in Tennessee. Their objective is to serve as the primary means of disseminating academic research to the public in an effort to improve quality of life through education. These agents work closely with livestock producers and were considered an asset for this project. Agents were contacted via e-mail and/or phone to determine interest in participating in the study (n = 50 agents). Twenty-six agents (52%) were willing to participate and subsequently sent a training video demonstrating the sampling methods employed for this study (Theuret and Trout Fryxell 2016). Agents were sent collection kits with the following items: Thermo Scientific Nunc 15-ml tubes (ThermoFisher, Waltham, MA) filled with approximately 7.5 ml of 80% ethanol, data sheets, and producer participation agreement forms. Additionally, Extension agents were provided labels and UT biological safety officer approved instructions for shipping samples in ethanol. Of the agents initially interested, nine (34.6% of interested investigators) reported and provided collection data. Use of each Extension agent as a tick scout was ideal because of their location, connection with local producers, rapid ability to respond to a potential threat, and their yearly training events.

In Tennessee, there are 47 facilities used for livestock auctions. Of these, 27 (57%) were contacted, with six (22% of contacted) willing to participate. These included three locations in the Interior Plateau (Tradition Livestock Services [35.895403, −86.38175], Warren County Livestock [35.709283, −85.791516]) and Dickson County Livestock Auction [36.023889, −87.341512]), two locations in the Mississippi Plains (Somerville Livestock Auctions [35.289827, −89.36078], and Scott's Hill Livestock Auction [35.51478, −88.238319]), and one location in the Ridge and Valley (East Tennessee Livestock Center [35.633839, −84.437595]). These locations held weekly auctions of cattle and calves, in addition to other livestock including pigs and goats. Use of each auction as a tick-check station was ideal because of their location, frequent use by a variety of local producers for weekly events, and established facilities, procedures, and methods.

Tick Collections From Cattle Hosts

Ticks were collected directly from cattle run through a chute to maximize the efficiency of collections and to protect the safety of both the investigator and the animal. The greater of 25% of the total herd size or 10 animals were sampled to capture ticks and avoid reducing the efficiency of the husbandry practices of the producer. For example, herds of less than 40 cattle sampled 10 animals, whereas a herd of 45 cattle sampled 12 cattle (11.25 rounded up). Collections were performed based on the schedule of the respective producer (herd manager) and were typically done concurrently with standard husbandry practices: vaccinations, pregnancy checks, ear tag insertions, and aging. Cattle were scratched (investigator used hands for tactile detection) and visually checked, with special attention to the ears, head, neck, tail, and underside of the tail, as these sites have been shown to be common attachment sites of ticks (Gladney et al. 1974, Barnard 1981, Barnard et al. 1982, Bloemer et al. 1988) and are safe for the inspector. Animals were sampled for a maximum of 5 min to minimize animal stress. Collected ticks were placed into a vial containing 80% ethanol, with one vial used per animal. Any ticks found on cattle or provided to us that were not part of the sampled group were considered opportunistically collected. At all collection sites, any cattle that posed a threat to the safety of itself or the investigator were not sampled. Information about each animal was recorded, including the ear tag number, breed, and age.

Tick Identification

All collected ticks were identified to species, life-stage, and sex using dichotomous keys (Sonenshine 1979). Following identification, ticks were placed into newly labeled vials of 80% ethanol for storage. Two variables used for statistical analysis were infestation prevalence (defined as the percentage of cattle within a sampled group that were infested with ticks) and tick burden (the mean number of ticks found on infested animals). No opportunistically collected ticks were used for statistical analyses. These variables along with traditional descriptive variables (e.g., mean no. ticks) were calculated for each species and the total. Data were visualized using ArcGIS (v 10.3.1) (ESRI 2011) to map tick collection sites, infestation prevalence, and tick burden. For all tests conducted, *Ixodes scapularis* (Say) (Acari: Ixodidae) collections were excluded from analyses because this species was rarely captured.

We also investigated co-occurrence on an animal (when two species occur together on the same host). Co-occurrence rates were compared using the Cole's index (C7) of interspecific association (Cole 1949). Positive values indicate a mutualistic relationship, negative values indicate competition, and numbers near zero indicate no association (neutral). Analyses were conducted on the spring and summer cattle collections using a Chi-square analysis to determine significance of association (α = 0.05).

Seasonal and Regional Effect

To determine seasonal and regional effects of tick prevalence and tick burden of cattle, we used a PROC GLM in SAS software 9.4 (Cary Institute, NC). This suite of tests included a MANOVA for multiple comparisons, ANOVA for species comparisons, and LSM separation adjusted for multiple comparisons using Tukey-Kramer. Response variable data were ranked transformed to satisfy the assumption of normality and equal variance required by the model. Seasons were defined by calendar month as spring (March, April, May), summer (June, July, August), and fall (September, October, November), for ease of interpretation by cattle producers. This included collections from three RECs (n = 604 animals; 201.33 ± 72.47 animals per season) at least once during these periods. Regions were defined according to the regions of the University of Tennessee Extension Service (western, middle, and eastern), and included collections from three RECs (n = 604 animals; 201.33 ± 72.47 animals per season), six livestock auctions (n = 419 animals; 69.83 ± 38.87 animals per season), and nine Extension agents at 21 collection sites (n = 374 animals; 17.81 ± 2.34 animals per season). For this analysis, only spring and summer collections were used; fall and winter collections were excluded because only four ticks were collected in fall and winter combined. Significance for the PROC GLM was determined at α = 0.05.

Spatial analysis was performed using SatScan (v 9.4.2) (Kulldorff 2015) to detect both high and low rates of infestation clusters. The parameters of this analysis require the size of the population at risk, the number of cases, and geographic coordinates. For this, the number of cattle sampled at a location was used as the population, with the number of cattle infested as the cases. A circular window with a radius equal to 50% of the cattle population size was used with no geographical overlap between windows. A Discrete Poisson model (Kulldorff 1997) was chosen because it is not sensitive to changing population sizes, a common occurrence in this study resulting from differences in the number of cattle sampled and herd size. Relative risk values are reported; with values <1 indicating decreased risk compared to baseline and values >1 indicating increased risk. For both analyses, the alpha level was α = 0.05. Again, fall and winter collections were excluded from analysis. Clustering results were displayed in ArcGIS (v10.3.1) (ESRI 2011).

Sites for Invasive Monitoring

To determine which collection method (RECs, extension collections [EXT], and/or auctions) would be best for future tick monitoring opportunities we compared infestation prevalence and tick burden from collections in the peak collection periods (spring and summer). This was used to make comparisons between collection sources due to greater temporal overlap in collections. Likewise, when investigating sex and age of animals as risk factors for tick parasitism animals were chosen from among regions that were not statistically different and from spring and summer. Significance for the PROC GLM was determined at $\alpha = 0.05$.

Results

Tick Collections

A total of 740 ticks (Acari: Ixodidae) were collected from cattle consisting of four species (Table 1). A majority (77.2%) of the collection were *Amblyomma americanum* (L.) (573 specimens) of which 61.6% were females, 31.4% were males, and 6.6% were nymphs. *Amblyomma maculatum* Koch comprised 16.8% of the collection (125 specimens) of which 84.8% were males and 15.2% were females. *Dermacentor variabilis* (Say) comprised 4.7% of the collection (35 specimens) of which 60.0% were female and 40.0% were male. The remaining 1.2% were three *I. scapularis* (adults) and four specimens missing key morphological features that made them unidentifiable using dichotomous keys. Due to our wide collection, some specimens were opportunistically collected and they included 53 *A. maculatum* (34 females and 19 males) and 35 *A. americanum* (24 females and 11 males); as mentioned, these were not used in any analyses.

Most cattle sampled during spring and summer were not infested with ticks (1,094 cattle; 78.3% tick-free) and if an animal was infested with ticks it was typically infested with only one species (285 cattle; 94.1%). Consequently, we rarely identified two different tick species co-occurring (simultaneously feeding) on the same animal (Table 2). We identified co-occurrence on 22 different animals (1.6% of sampled animals) and we never observed three different species on the same host. Cole's index of association for *A. americanum* and *D. variabilis* was 0.299 ± 0.0799 ($\chi^2 = 14.09$; $P = 0.0002$) indicating a significantly positive interspecific relationship. Whereas, Cole's index of association for *A. americanum* and *A. maculatum* was −0.437 ± 0.3350 ($\chi^2 = 1.695$; $P = 0.1929$) and for *A. maculatum* and *D. variabilis* was 0.039 ± 0.0329 ($\chi^2 = 1.242$; $P = 0.2650$) indicating no significant relationship between the different co-occurrence species.

Knowing these tick species mate on their hosts, we also compared intraspecific interactions. Cole's index of association for *A. americanum* adults and nymphs was 0.686 ± 0.0825 ($\chi^2 = 69.17$; $P < 0.0001$) and for males and females it was 0.351 ± 0.0341 ($\chi^2 = 105.54$; $P < 0.0001$) indicating all nymph, male, and female *A. americanum* were significantly associated together on cattle. This was also significant for *A. maculatum* males and females; their Cole's index of association was 0.606 ± 0.0443 and positively associated with one another ($\chi^2 = 187.21$; $P < 0.0001$).

Effects of Season and Region

Total

Infestation prevalence ($F = 9.54$; df = 2; $P = 0.0021$; Table 3) and burden ($F = 11.16$; df = 2; $P = 0.0011$; Table 4) were different between fall and spring collections ($P < 0.005$). Both infestation prevalence ($F = 0.16$; df = 2; $P = 0.8488$; Fig. 2) and burden ($F = 0.30$; df = 2; $P = 0.7408$; Fig. 3) were not significant between regions of Tennessee. One cluster encompassing nine locations in middle and western Tennessee was significant for high tick infestation rates ($P < 0.0001$) with a relative risk of 3.01 (Fig. 4). There were also two clusters encompassing 11 locations in middle and eastern, and one in western, Tennessee were significant for low rates of infestation ($P < 0.001$) with relative risk ranging from 0.19 to 0. Locations for both high and low rate clusters comprised all three collection source types (RECs, auctions, extension collections).

Amblyomma americanum

For *A. americanum,* neither infestation prevalence ($F = 1.59$; df = 2; $P = 0.2361$) or burden ($F = 1.96$; df = 2; $P = 0.1756$) were significantly impacted by season. We observed the same insignificant patterns in infestation prevalence ($F = 0.13$; df = 2; $P = 0.8811$) and burden ($F = 0.85$; df = 2; $P = 0.4375$) between regions. Further spatial analysis revealed one high rate cluster comprised of four locations in middle Tennessee that had significant clusters of infestation for *A. americanum* ($P < 0.001$) with a relative risk of 3.82. This cluster included an auction and several extension collections. Four significant low rate clusters ($P < 0.05$) with relative risk ranging from 0.092 to 0 were detected in neighboring locations comprised of RECs and extension locations.

Amblyomma maculatum

Season had a significant effect on infestation prevalence ($F = 6.82$; df = 2; $P = 0.0078$) and burden ($F = 6.68$; df = 2; $P = 0.0084$), with fall lower than spring ($P < 0.05$). Additionally, both infestation

Table 1. *Amblyomma americanum*, *Amblyomma maculatum*, and *Dermacentor variabilis* were found parasitizing cattle in Tennessee

Tick species	Life-stage	No. ticks	No. animals	Mean (±SEM)	Infestation prevalence (%)	Tick burden
Amblyomma americanum	Nymph	40	32	0.02 ± 0.004	1.76	1.25
	Male	180	109	0.01 ± 0.010	5.99	1.65
	Female	353	185	0.19 ± 0.020	10.18	1.91
	Total	573	252	0.32 ± 0.030	13.87	2.27
Amblyomma maculatum	Nymph	0	0	0	0	0
	Male	106	35	0.06 ± 0.010	1.93	3.03
	Female	19	13	0.01 ± 0.003	0.72	1.46
	Total	125	40	0.07 ± 0.020	2.20	3.13
Dermacentor variabilis	Nymph	0	0	0	0	0
	Male	14	14	0.01 ± 0.002	0.77	1
	Female	21	21	0.01 ± 0.002	1.16	1
	Total	35	33	0.02 ± 0.003	1.82	1.06

Additional specimens collected from sampled cattle include three *Ixodes scapularis* and four tick specimens (0.5%) missing key morphological features which made them unidentifiable using dichotomous keys.

Table 2. Co-occurrence of two different tick species[a] was rarely documented on cattle (1.6% of sampled animals), but significant interspecific and intraspecific competition did occur

Dominant species	Co-species	Number of cattle: Both present	Only dominant species	Only co-occurring species	Both absent	Cole's index (C_7 ± SE)
Interspecific competition						
Amblyomma americanum	*Amblyomma maculatum*	4	244	36	1,113	−0.437 ± 0.3350 (*P* = 0.1929)
Amblyomma americanum	*Dermacentor variabilis*	14	234	19	1,130	0.300 ± 0.0799 (***P* = 0.0002**)
Amblyomma maculatum	*Dermacentor variabilis*	2	38	31	1,326	0.039 ± 0.0329 (*P* = 0.2650)
Intraspecific competition						
Amblyomma americanum adults	*Amblyomma americanum* nymphs	23	225	8	1,141	0.686 ± 0.0825 (***P* < 0.0001**)
Amblyomma americanum females	*Amblyomma americanum* males	46	130	56	1,165	0.351 ± 0.0341 (***P* < 0.0001**)
Amblyomma maculatum females	*Amblyomma maculatum* males	8	27	5	1,357	0.606 ± 0.0443 (***P* < 0.0001**)

P values that are <0.05 are considered significant and are bolded.
[a]*Ixodes scapularis* was found co-occurring on one animal with *D. variabilis* and another animal with *A. maculatum*. These interactions occurred only once each.

Table 3. Overall infestation prevalence for the study was 16.9% for sampled cattle[a]

Variable of interest (*n* = no. cattle)	*Amblyomma americanum*	*Amblyomma maculatum*	*Dermacentor variabilis*	Overall
Seasonal effect				
Spring (*n* = 297)	6.5 ± 3.85	16.2 ± 10.67a	2.5 ± 1.16	23.6 ± 10.01a
Summer (*n* = 307)	9.1 ± 9.09	0.9 ± 0.93ab	1.6 ± 0.93	10.9 ± 8.54ab
Fall (*n* = 194)	0	0b	0	0b
Statistic *F* (*P*)	1.59 (*0.2361*)	**6.82 (*0.0078*)***	3.54 (*0.0550*)	**9.54 (*0.0021*)***
Regional effect				
Western (*n* = 362)	20.0 ± 4.47	3.9 ± 1.88a	3.6 ± 1.75	24.9 ± 5.49
Middle (*n* = 628)	26.2 ± 7.45	1.2 ± 1.20b	1.6 ± 1.13	27.4 ± 7.33
Eastern (*n* = 407)	22.3 ± 18.87	0ab	1.1 ± 0.56	23.3 ± 18.38
Statistic *F* (*P*)	0.13 (*0.8811*)	**4.83 (*0.0161*)***	2.10 (*0.1416*)	0.16 (*0.8488*)
Site effect				
REC (*n* = 604)	7.7 ± 6.99	9.5 ± 7.38a	2.0 ± 0.32	17.6 ± 7.51
EXT (*n* = 374)	27.4 ± 7.18	0b	1.9 ± 1.20	27.9 ± 7.18
Auction (*n* = 419)	21.9 ± 6.48	3.9 ± 2.20a	2.6 ± 1.04	25.6 ± 7.11
Statistic *F* (*P*)	0.52 (*0.5985*)	**18.3 (*<0.0001*)***	**4.81 (*0.0163*)***	0.19 (*0.8271*)

[a]Statistics are reported as the *F* value and respective *P* value as *F*(*P*). *P* values that are significant are bolded and denoted by (*). Mean values are calculated from raw data and do not reflect rank-transformed data. Mean values within a column with different lower-case letters are significantly different at *P* < 0.05.

prevalence ($F = 4.83$; df = 2; $P = 0.0161$) and burden ($F = 4.53$; df = 2; $P = 0.0201$) were shown to be significant between regions. Least-squared means demonstrated that western Tennessee was significantly different from middle Tennessee in both infestation prevalence ($P = 0.0176$) and burden ($P = 0.0222$) and both of these regions were not significantly different from eastern Tennessee for either variable. Cluster analysis showed one auction and one REC along the border of middle and western Tennessee were a cluster of high infestation rates ($P = 1.0 \times 10^{-17}$) with a relative risk of 24.85. Several locations in middle and eastern Tennessee formed a significant cluster of low rates of infestation ($P = 7.6 \times 10^{-11}$) with a relative risk of 0 and were comprised of all three collection source types.

Dermacentor variabilis

For *D. variabilis*, season was not significantly associated with infestation prevalence ($F = 3.54$; df = 2; $P = 0.0550$) or burden ($F = 3.55$; df = 2; $P = 0.0546$). Like the patterns seen in total and *A. americanum*, infestation prevalence ($F = 2.10$; df = 2; $P = 0.1416$) and burden ($F = 2.68$; df = 2; $P = 0.0868$) were not significant between regions. One location in western Tennessee was shown to be a significant high cluster for *D. variabilis* ($P = 0.039$) that had a relative risk of 6.25. There were no locations that were considered significant low clusters for *D. variabilis*.

Sites for Invasive Monitoring

We attempted to compare phenotypic traits of the animals including sex and age, but all comparisons were insignificant ($P > 0.05$). There was a significant effect due to site type ($F = 6.68$; df = 16; $P < 0.0001$), which was driven by differences observed in *A. maculatum* and *D. variabilis*. The infestation prevalence ($F = 18.33$; df = 2; $P < 0.0001$) and burden ($F = 18.58$; df = 2; $P < 0.0001$) of *A. maculatum* were greatest at the auctions and RECs ($P < 0.001$). For *D. variabilis*, burden ($F = 11.13$; df = 2; $P = 0.0003$) was significantly greater at the auctions and RECs compared to extension collections ($P < 0.05$).

Table 4. The overall tick burden for the study was 2.4 for sampled cattle[a]

Variable of interest (n = no. cattle)	*Amblyomma americanum*	*Amblyomma maculatum*	*Dermacentor variabilis*	Overall
Seasonal effect				
Spring (n = 297)	0.6 ± 0.29	1.7 ± 0.69a	0.6 ± 0.24	2.0 ± 0.64a
Summer (n = 307)	0.4 ± 0.35	0.3 ± 0.25ab	0.5 ± 0.29	0.9 ± 0.31ab
Fall (n = 194)	0	0b	0	0b
Statistic F (P)	1.96 (*0.1756*)	**6.68 (*0.0084*)***	3.55 (*0.0546*)	**11.16 (*0.0011*)***
Regional effect				
Western (n = 362)	2.1 ± 0.75	0.6 ± 0.22a	0.6 ± 0.20	1.8 ± 0.54
Middle (n = 628)	1.5 ± 0.39	0.3 ± 0.22b	0.2 ± 0.09	1.7 ± 0.42
Eastern (n = 407)	1.5 ± 0.19	0ab	0.7 ± 0.37	1.5 ± 0.18
Statistic F (P)	0.85 (*0.4375*)	**4.83 (*0.0161*)***	2.68 (*0.0868*)	0.30 (*0.7408*)
Site effect				
REC (n = 604)	0.9 ± 0.45	1.8 ± 1.29a	1.1 ± 0.07a	2.4 ± 1.01
EXT (n = 374)	1.5 ± 0.37	0b	0.1 ± 0.08b	1.5 ± 0.37
Auction (n = 419)	2.4 ± 0.84	0.7 ± 0.23a	0.7 ± 0.22a	2.1 ± 0.60
Statistic F (P)	1.62 (*0.2165*)	**18.58 (<*0.0001*)***	11.13 (*0.0003*)*	1.81 (*0.1829*)

[a]Statistics are reported as the F value and respective P value as $F(P)$. P values that are significant are bolded and denoted by (*). Mean values are calculated from raw data and do not reflect rank-transformed data. Mean values within a column with different lower-case letters are significantly different at $P < 0.05$.

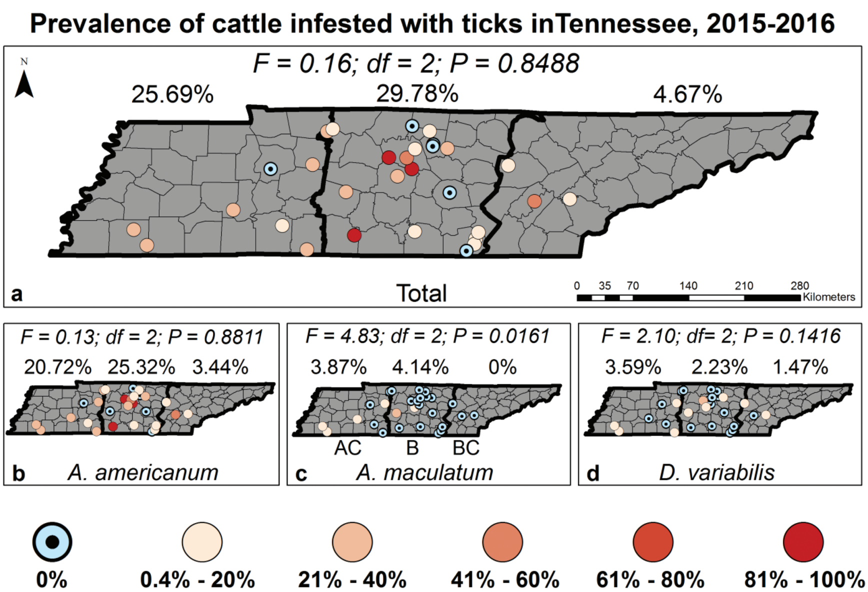

Fig. 2. Infestation prevalence of cattle infested with ticks in Tennessee. Values shown are calculated from raw data and do not represent rank-transformed data. Infestation prevalence varied by the species total (a) and each tick species; *Amblyomma americanum* (b), *Amblyomma maculatum* (c), and *Dermacentor variabilis* (d). Region was only significant for *A. maculatum* infestation prevalence, with regions with different upper-case letters being significantly different at $P < 0.05$.

Discussion

Results of this study confirm that *A. americanum*, *A. maculatum*, and *D. variabilis* are pests of cattle in Tennessee (Pompo et al. 2016). One difference in these two studies is that in this survey *I. scapularis* was also identified as a parasite of cattle and was completely absent from the previous study. Adult *I. scapularis* were previously documented as a pest of cattle with a seasonal activity ranging from October through April (Bishopp and Trembley 1945, Harris 1959, Drummond 1967, Barnard 1981). Our results corroborate these findings, *I. scapularis* were captured in low numbers (n = 3) in winter and early spring. Therefore, the absence of *I. scapularis* from Pompo et al. (2016) is likely due to the summer sampling employed in their survey, which would have missed the window of activity for adult *I. scapularis*.

The most common tick species collected was *A. americanum*. This species is abundant with a wide geographic range; it was captured at 23 sites using all collection types and has high infestation prevalence and tick burden throughout the spring and summer. These characteristics make *A. americanum* a primary ectoparasite of Tennessee cattle. Previously, 15 female *A. americanum* per animal was the injury threshold for pre-weaned beef cattle (Barnard 1985). None of the animals sampled in this study had more than the threshold (maximum was 11 female *A. americanum* per single animal) indicating these tick populations were not at damaging levels; however, we could only sample from a limited portion of the animal's body surface unlike Barnard (1985) who performed whole-body inspections. Given this consideration, it is possible that infested herds had more ticks than we could capture, and thus producers in

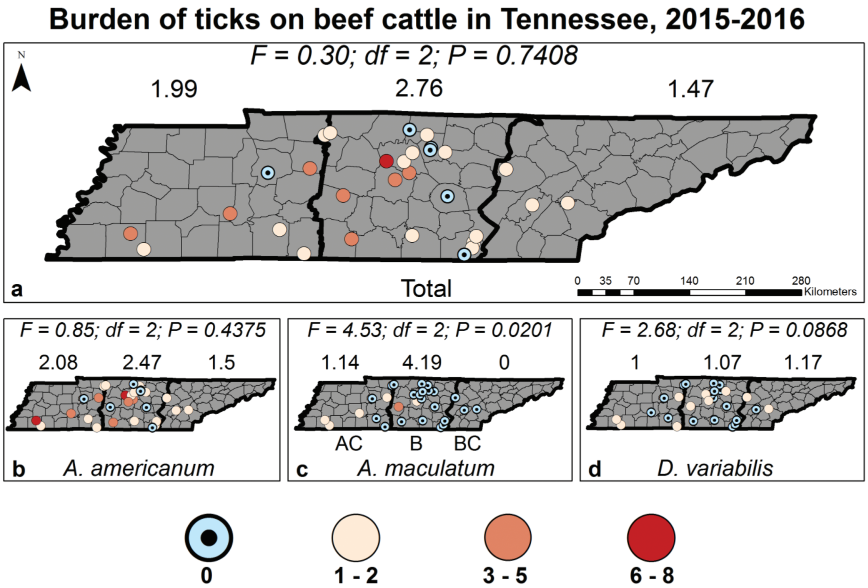

Fig. 3. Burden of ticks on cattle in Tennessee. Values shown are calculated from raw data and do not represent rank-transformed data. Burden varied by the species total (a) and each tick species; *Amblyomma americanum* (b), *Amblyomma maculatum* (c), and *Dermacentor variabilis* (d). Region was only significant for *A. maculatum* burden, with regions with different upper-case letters being significantly different at $P < 0.05$.

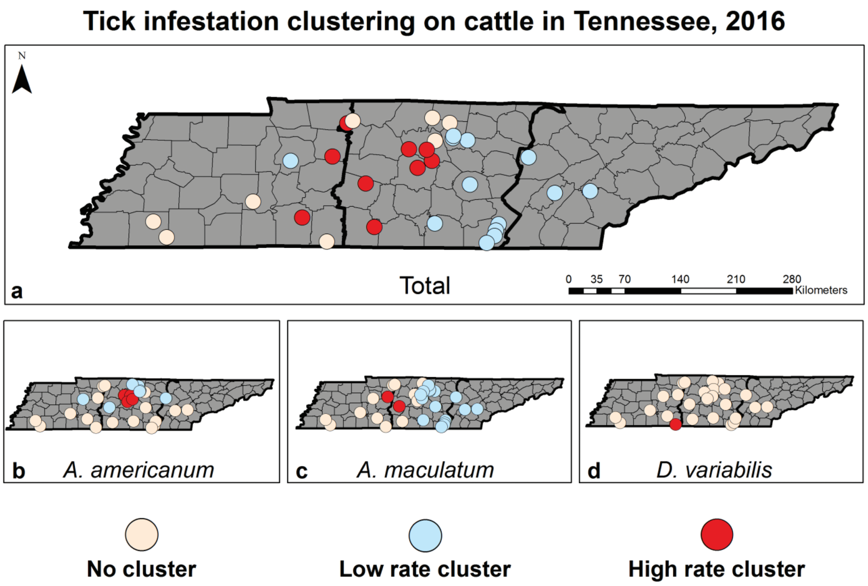

Fig. 4. Spatial cluster analysis of tick infestation on cattle in Tennessee. High-rate clusters were found for the species total (a) and each tick species; *Amblyomma americanum* (b), *Amblyomma maculatum* (c), and *Dermacentor variabilis* (d). Low-rate clusters were found for each species except *D. variabilis.*

Tennessee may already be suffering economic losses due to *A. americanum* feeding damage.

Conversely, *D. variabilis* were collected from only 10 locations, both infestation prevalence and burden were low and not impacted by either season or region, and little geographic clustering was observed. Previous survey results found *D. variabilis* in 40 of 49 sampled counties in Tennessee, suggesting that it has a wide geographic range (Cohen et al. 2010). Knowledge of the geographic range of this pest is important because *D. variabilis* is a biological vector of *A. marginale*, and its distribution may indicate geographic range of this pathogen. A high proportion of Tennessee beef cattle (56%) tested between 2002 and 2012 were infected with *A. marginale*, with 10.53% of samples positive in 2013 (Whitlock et al. 2014). Since this species is widespread, but has a low infestation prevalence and low tick burden, this may explain Tennessee's relatively low BA rates (e.g., there are not enough infected ticks to cause infection at this time). Furthermore, knowledge of the phenology and regional distribution of *D. variabilis* is important for veterinarians to prescribe medication under new regulations outlined by the veterinary feed directive. The directive dictates that the supervision of a veterinarian who has a veterinarian-client patient relationship with the producer is necessary to administer medicated feeds to herds, with medications only given to treat or prevent disease; the latter case should only occur if the veterinarian is able to determine that contracting an illness is likely (FDA 2012, 2013, 2015). Future studies should determine the infection rates of *A. marginale* in *D. variabilis* in different BA infection areas (low to high risk) to elucidate the risk to cattle and to assist veterinarians in making informed decisions about prescription of feed-through antibiotics within the boundaries of the directive.

We collected *A. maculatum* from six sites within a restricted distribution in middle Tennessee; none were collected in eastern Tennessee. Originally distributed along the Gulf Coast region of the United States (Texas, Louisiana, Mississippi, Alabama, Georgia, and Florida), populations of *A. maculatum* have expanded via cattle movement into Oklahoma and Kansas (Teel et al. 2010) with only occasional collections of *A. maculatum* in western Tennessee (Bishopp and Trembly 1945, Durden and Kollars 1992, Mays et al. 2016). This tick has also been sporadically collected within the middle Tennessee region. A single *A. maculatum* was captured in Marshall County Tennessee (Pompo et al. 2016), while a single tick from both Perry and Decatur Counties was found during a statewide tick survey (Cohen et al. 2010). In this study, we captured 160 adult *A. maculatum* in Maury County, which is within 100 miles of Perry, Decatur, and Marshall counties. Previous collections of this tick within Tennessee were attributed to accidental introductions either through livestock importation (Bishopp and Trembly 1945) or movement of bird hosts (Durden and Kollars 1992). Our results, combined with recent findings by other authors, suggest that the range of *A. maculatum* is expanding in western and middle Tennessee.

Given its recent expansion into the state, *A. maculatum* should be considered a 'model' invasive tick species. Results from our investigation into which sources would be best for invasive monitoring revealed that cattle infested with *A. maculatum* had high prevalence and burden at the RECs and auction collections. Within Tennessee the RECs and auctions should continue to be used as monitoring resources, with the RECs acting as sentinels that can detect established populations of invasive species and the auctions as a check station for potential invasions. Livestock auctions should be the primary means of monitoring for invasive ticks. First, the number of new cattle moving into these locations is greater compared to the RECs, potentially increasing the likelihood of capturing invasive ticks. Especially useful would be auctions located at the borders of Tennessee, which would have a greater chance of sampling imported cattle crossing state lines. Second, the number of auction locations in the state is far greater (>40) compared to the number of university-operated RECs with cattle (7), which would allow for greater regional spread in collections. Lastly, although the RECs willingly cooperated with sampling efforts in this study, collections were scheduled to coincide with other husbandry practices (ear tagging, vaccinations, pregnancy checking, etc.) which are performed a limited number of times annually. The auctions, if they offer pregnancy checking at their facilities, have regular inspections of cattle, with many of the locations in this study conducting auctions once a week. Therefore, this could offer a weekly monitoring schedule for tick activity. Future surveillance efforts using these guidelines could offer the opportunity to increase the resolution of the geographic distribution and seasonal phenology of ticks and serve as an effective means for monitoring for invasive ticks.

Interestingly, *A. americanum* and *D. variabilis* were found co-occurring on 1.0% ($n = 14$) of sampled animals but still had a significant positive co-infestation relationship, meaning that when *D. variabilis* is found on a host it is likely that *A. americanum* will also be present. Several factors can explain this positive relationship, including similar host use, overlapping geographic distribution, and matching temporal patterns of activity; however, we believe it is the questing environment and use of hosts during immature stages that accounts for this relationship. Further investigations into the wildlife and plant community around cattle are warranted and would likely support our claim. Previous work in Tennessee confirmed that *D. variabilis* and *A. americanum* are commonly collected through drag sampling and mammal trapping (Cohen et al. 2010) and are both parasites of Tennessee cattle (Pompo et al. 2016). Lastly, several publications demonstrated that the seasonal activity of *A. americanum* (Davidson et al. 1994, Jackson et al. 1996) and *D. variabilis* (Burg 2001) occurs primarily in spring with adults disappearing by August. The finding that these two species co-infest cattle is important for two reasons. The first is the potential for *D. variabilis* to act as an infestation indicator for *A. americanum*, which may be useful for determining if the economic threshold has been surpassed, although more research would be required to elucidate the relationship between *D. variabilis* and *A. americanum* densities on cattle. Second, although *A. americanum* is not considered a biological vector of *A. marginale*, it could nonetheless play an important role in pathogen transmission by suppressing the host immune response (Wikel and Whelen 1986, Wikel et al. 1994, Wikel 1999), allowing for infection via *D. variabilis* feeding.

Importantly, several pathogens and invasive ticks are threatening the U.S. cattle industry. As mentioned, the distributions of *A. americanum* and *D. variabilis* may serve as predictors for *A. marginale* distributions and *A. maculatum's* distribution may serve as a predictor for *E. ruminantium* distribution. Several southern U.S. states could be invaded by multiple threats, including the Texas Cattle fever ticks (*R. microplus* and *R. annulatus*) that transmit the agents of Texas Cattle fever (*B. bovis* and *B. bigemina*) and the Bont ticks (*A. variegatum* and *Amblyomma hebraeum* Koch (Acari:Ixodidae)) which can both transmit the agent of HW (*E. ruminantium*).

The finding of *H. longicornis*-infested sheep in New Jersey and *H. longicornis*-infested calves in Virginia are poignant reminders of the importance of tick and pathogen surveillance, especially for invasive tick and pathogen complexes threatening the U.S. cattle industry (Anonymous 2018, Rainey et al. 2018). This three-host tick species is found in several countries including: Korea, Japan, China, USSR (former), Australia, and New Caledonia (Heath et al. 2011) and is capable of damaging cattle through transmission of *B. ovata* and *T. orientalis* (Fujinaga 1981, Sivakumar et al. 2012). The primary threat to cattle is irritation and blood loss via feeding, which can occasionally cause death in calves (Hoogstraal et al. 1968). In New Zealand where this species is invasive, the greatest economic impact is to the pelt and hide industry (Heath 1994), and dairy industry with up to 25% reduction in production noted in dairy cattle (Cane 2010). This species has several attributes that likely contribute to its success as an invader. The wide host range of *H. longicornis* (Tenquist and Charleston 2001, Fonseca et al. 2017) means that wildlife and other animals could sustain tick populations and therefore serve as reservoirs for continual infestation of livestock and introduction of ticks into new areas. Additionally, invasive populations of *H. longicornis* are known to reproduce parthenogenically (Hoogstraal et al. 1968) meaning one female tick can easily establish a new population. Rainey et al. (2018) observed that multiple life-stages infested the New Jersey sheep and that only one male tick was found, suggesting that the infestation came from a parthenogenic population. They also stated that the sheep had no travel history outside of the state, which opens the possibility that this tick species is already established in this area. Finding H. longicornis-infested animals in both adjacent, regional, and distant states on livestock and wildlife supports this claim (Anonymous 2018). Applying our results, officials should use livestock auctions as tick-check stations to monitor spread and establishment, while producers should be vigilant about checking their own, their newly purchased, and/or moved cattle for ticks.

Early detection of *H. longicornis* and other tick species capable of affecting the cattle industry in the United States (*R. microplus*, *R. annulatus*, *A. variegatum*) is paramount to effective quarantine strategies and determination of geographic distribution of associated pathogens. Future work should expand the proposed surveillance strategy from Tennessee into other states to protect the U.S. cattle, and other livestock, industries. Establishing a multi-state collaborative system would permit early detection of tick threats and make concerted eradication and quarantine efforts possible.

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References Cited

Anonymous. 2018. Longhorned tick found on cattle on a Virginia farm. Outbreak News Today. Accessed 23 May 2018. Available from http://outbreaknewstoday.com/longhorned-tick-found-cattle-virginia-farm-43455/

Barnard, D. R. 1981. Seasonal activity and preferred attachment sites of *Ixodes scapularis* (Acari: Ixodidae) on cattle in southeastern Oklahoma. J. Kans. Entomol. Soc. 54: 547–552.

Barnard, D. R. 1985. Injury thresholds and production loss functions for the lone star tick, *Amblyomma americanum* (Acari: Ixodidae), on pastured, preweaner beef cattle, *Bos taurus*. J. Econ. Entomol. 78: 852–855.

Barnard, D., B. Jones, and G. Rogers. 1982. Sites of attachment of *Amblyomma americanum* to cattle. Ann. Entomol. Soc. Am. 75: 222–223.

Bishopp, F., and H. L. Trembley. 1945. Distribution and hosts of certain North American ticks. J. Parasitol. 31: 1–54.

Bloemer, S. R., R. H. Zimmerman, and K. Fairbanks. 1988. Abundance, attachment sites, and density estimators of lone star ticks (Acari: Ixodidae) infesting white-tailed deer. J. Med. Entomol. 25: 295–300.

Burg, J. G. 2001. Seasonal activity and spatial distribution of host-seeking adults of the tick *Dermacentor variabilis*. Med. Vet. Entomol. 15: 413–421.

Burridge, M., R. Norval, and S. Deem. 1992. Heartwater: a potential threat outside Africa, pp. 155–162. In B. Osburn, G. Castrucci, and C. Schore (eds.), Proceedings, World Association of Veterinary Microbiologists, Immunologists, and Specialists in Infectious Diseases (W.A.V.M.I) 12th International Symposium: New and Emerging Infectious Diseases, 8-12 September 1992, Davis, CA. University of California Davis, Davis, CA.

Busch, J. D., N. E. Stone, R. Nottingham, A. Araya-Anchetta, J. Lewis, C. Hochhalter, J. R. Giles, J. Gruendike, J. Freeman, G. Buckmeier, et al. 2014. Widespread movement of invasive cattle fever ticks (*Rhipicephalus microplus*) in southern Texas leads to shared local infestations on cattle and deer. Parasit. Vectors. 7: 188.

Cane, R. 2010. Profile: *Haemaphysalis longicornis* Neumann 1901. New Zealand Biosecure Entomology Laboratory. Available from https://www.smsl.co.nz/site/southernmonitoring/files/NZB/Ha%20longicornis%20Profile.pdf.

Cohen, S. B., J. D. Freye, B. G. Dunlap, J. R. Dunn, T. F. Jones, and A. C. Moncayo. 2010. Host associations of *Dermacentor, Amblyomma*, and *Ixodes* (Acari: Ixodidae) ticks in Tennessee. J. Med. Entomol. 47: 415–420.

Cole, L. C. 1949. The measurement of interspecific association. Ecology 30: 411–424.

Davidson, W. R., D. A. Siefken, and L. H. Creekmore. 1994. Seasonal and annual abundance of *Amblyomma americanum* (Acari: Ixodidae) in central Georgia. J. Med. Entomol. 31: 67–71.

Deem, S. L. 1998. A review of heartwater and the threat of introduction of *Cowdria ruminantium* and *Amblyomma* spp. ticks to the American mainland. J. Zoo Wildl. Med. 29: 109–113.

Dietrich, R. A., and L. G. Adams. 2000. Potential animal health concerns relative to cattle fever ticks, classical swine fever, and bovine brucellosis, with special emphasis on Texas. Texas Agricultural Experiment Station, Texas A&M University System, College Station, TX.

Dikmans, G. 1950. The transmission of Anaplasmosis. Am. J. Vet. Res. 11: 5–16.

Drummond, R. 1967. Seasonal activity of ticks (Acarina: Metastigmata) on cattle in southwestern Texas. Ann. Entomol. Soc. Am. 60: 439–447.

Durden, L., and T. Kollars Jr. 1992. An annotated list of the ticks (Acari: Ixodoidea) of Tennessee, with records of four exotic species for the United States. Bull. Soc. Vector. Ecol. 17: 125–131.

ESRI. 2011. Release 10. Environmental Systems Research Institute, Redlands, CA.

(FDA) U.S. Food and Drug Administration. 2012. Guidance for Industry: the judicious use of medically important antimicrobial drugs in food-producing animals. #209. Food and Drug Administration, Rockville, MD. Available from https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM216936.pdf

(FDA) U.S. Food and Drug Administation. 2013. Guidance for industry: new animal drugs and new animal drug combination products administered in or on medicated feed or drinking water of food-producing animals: recommendations for drug sponsors for voluntarily aligning product use conditions with GFI #209. #213. Food and Drug Administration, Rockville, MD. Available from https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM299624.pdf

(FDA) U.S. Food and Drug Administation. 2015. Veterinary feed directive. Fed. Reg. 80: 31708-31735. National Archives and Records Administration, Washington, DC. Available from https://www.gpo.gov/fdsys/pkg/FR-2015-06-03/pdf/2015-13393.pdf

Fonseca, D. M., A. Egizi, and J. Occi. 2017. Global Health: the tick that binds us all. Review of the biology and ecology of *Haemaphysalis longicornis* Neumann 1901. Center for Vector Biology. Rutgers, the State University of New Jersey. Available from https://fonseca-lab.com/research/global-health-the-tick-that-binds-us-all/. Accessed 23 May 2018.

Fujinaga, T. 1981. Bovine Babesiosis in Japan: clinical and clinico-pathological studies on cattle experimentally infected with *Babesia ovata*. Jpn. J. Vet. Sci. 43: 803–813.

Gladney, W., S. Ernst, and R. Grabbe. 1974. The aggregation response of the Gulf Coast tick on cattle. Ann. Entomol. Soc. Am. 67: 750–752.

Goodger, W. J., T. Carpenter, and H. Riemann. 1979. Estimation of economic loss associated with anaplasmosis in California beef cattle. J. Am. Vet. Med. Assoc. 174: 1333–1336.

Griffith, G.E., J.M. Omernik, and S. H. Azevedo. 1998. Ecoregions of Tennessee. U.S. Geological Survey, Reston, VA. Scale 1:940,000. Available from https://store.usgs.gov/assets/MOD/StoreFiles/Ecoregion/21632_tn_front.pdf

Harris, R. L. 1959. Biology of the black-legged tick. J. Kans. Entomol. Soc. 32: 61–68.

Heath, A. C. G. 1994. Ectoparasites of livestock in New Zealand. N.Z. J. Zoology. 21: 23–38.

Heath, A. C. G., R. L. Palma, R. P. Cane, and S. Hardwick. 2011. Checklist of New Zealand ticks (Acari: Ixodidae, Argasidae). Zootaxa. 2995: 55–63.

Hoogstraal, H., F. H. Roberts, G. M. Kohls, and V. J. Tipton. 1968. Review of *Haemaphysalis (kaiseriana) Longicornis* Neumann (resurrected) of Australia, New Zealand, New Caledonia, Fiji, Japan, Korea, and Northeastern China and USSR, and its parthenogenetic and bisexual populations (Ixodoidea, Ixodidae). J. Parasitol. 54: 1197–1213.

Jackson, L. K., D. M. Gaydon, and J. Goddard. 1996. Seasonal activity and relative abundance of *Amblyomma americanum* in Mississippi. J. Med. Entomol. 33: 128–131.

Jongejan, F., and G. Uilenberg. 2004. The global importance of ticks. Parasitology. 129(Suppl): S3–14.

Kocan, K. M., J. de la Fuente, E. F. Blouin, and J. C. Garcia-Garcia. 2004. *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne *Rickettsia*. Parasitology. 129(Suppl): S285–S300.

Kulldorff, M. 1997. A spatial-scan statistic. Commun. Stat. Theory Methods 26: 1481–1496.

Kulldorff, M. 2015. SaTScan TM v 9.4.2: software for the spatial and space-time statistics. Information Management Services, Inc. Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, MA.

Li, A. Y., R. B. Davey, R. J. Miller, and J. E. George. 2003. Resistance to coumaphos and diazinon in *Boophilus microplus* (Acari: Ixodidae) and evidence for the involvement of an oxidative detoxification mechanism. J. Med. Entomol. 40: 482–490.

Li, A. Y., R. B. Davey, R. J. Miller, and J. E. George. 2004. Detection and characterization of amitraz resistance in the southern cattle tick, *Boophilus microplus* (Acari: Ixodidae). J. Med. Entomol. 41: 193–200.

Mays, S. E., A. E. Houston, and R. T. Trout Fryxell. 2016. Specifying Pathogen Associations of *Amblyomma maculatum* (Acari: Ixodidae) in Western Tennessee. J. Med. Entomol. 53: 435–440.

McCallon, B.R. 1973. Prevalence and economic aspects of Anaplasmosis, pp. 1–3. In E.W. Jones (ed.), Proceedings, 6th National Anaplasmosis Conference, 19-20 March 1973, Las Vegas, NV. Heritage Press, Stillwater OK.

Merriman, G. M., L. K. Owens, P. K. Chung, J. B. McLaren, and C. S. Hobbs. 1966. Serological diagnosis and control of Anaplasmosis in Tennessee cattle. University of Tennessee Agricultural Experiment Station Bulletin. 400: 29pp.

Miller, R. J., R. B. Davey, and J. E. George. 2005. First report of organophosphate-resistant *Boophilus microplus* (Acari: Ixodidae) within the United States. J. Med. Entomol. 42: 912–917.

Neel, J. B. 2013. The Tennessee cattle industry and the Master Beef Producer Program, pp. 1–8. In Tennessee Master Beef Producer Manual. PB1722 (Original work published in 2004). UT Extension, The University of Tennessee, Knoxville, TN.

Pompo, K., S. Mays, C. Wesselman, D. J. Paulsen, and R. T. Fryxell. 2016. Survey of ticks collected from Tennessee cattle and their pastures for *Anaplasma* and *Ehrlichia* species. J. Parasitol. 102: 54–59.

Rainey, T., J. L. Occi, R. G. Robbins, and A. Egizi. 2018. Discovery of *Haemaphysalis longicornis* (Ixodida: Ixodidae) Parasitizing a Sheep in New Jersey, United States. J. Med. Entomol. 55: 757–759.

Ristic, M. 1977. Bovine anaplasmosis, pp 235–249. *In* **J. Kreier** (ed.), Parasitic protozoa, vol. 4. Academic Press, New York, NY.

Sivakumar, T., M. Tagawa, T. Yoshinari, A. P. Ybañez, I. Igarashi, Y. Ikehara, H. Hata, S. Kondo, K. Matsumoto, H. Inokuma, et al. 2012. PCR detection of *Babesia ovata* from cattle reared in Japan and clinical significance of coinfection with *Theileria orientalis*. J. Clin. Microbiol. 50: 2111–2113.

Sonenshine, D. E. 1979. Insects of Virginia. 13. Ticks of Virginia (Acari, Metastigmata). Virginia Polytechnic Institute Research Division Bulletin 139. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Teel, P. D., H. R. Ketchum, D. E. Mock, R. E. Wright, and O. F. Strey. 2010. The Gulf Coast tick: a review of the life history, ecology, distribution, and emergence as an arthropod of medical and veterinary importance. J. Med. Entomol. 47: 707–722.

Tenquist, J. D., and W. A. G. Charleston. 2001. A revision of the annotated checklist of ectoparasites of terrestrial mammals in New Zealand. J. Royal Soc. N.Z. 31: 481–542.

Theuret, D., and R. Trout Fryxell. 2016. Tick diversity survey [Digital Video]. Department of Entomology and Plant Pathology, University of Tennessee. Available from https://youtu.be/bHxvLfbsxSM.

(USDA-ERS) United States Department of Agriculture Economic Research Service. 2017. Cattle and Beef Statistics and Information. Available from https://www.ers.usda.gov/topics/animal-products/cattle-beef/statistics-information.aspx.

Vilsack, T., and C. Clark. 2014. 2012 Census of Agriculture: United States. United States Department of Agriculture, National Agricultural Statistics Service. Available from https://www.agcensus.usda.gov/Publications/2012/.

Wagner, G. G., P. Holman, and S. Waghela. 2002. Babesiosis and heartwater: threats without boundaries. Vet. Clin. North Am. Food Anim. Pract. 18: 417–30, vi.

Whitlock, B. K., J. A. Daniel, B. S. Harvey, J. K. Johnson, and J. F. Coetzee. 2014. Seroprevalence of bovine anaplasmosis in the southern U.S., pp. 157. *In* R. A. Smith (ed.), Proceedings, American Association of Bovine Practitioners, 18–20 September 2014, Albuquerque, NM. American Association of Bovine Practitioners, Ashland, OH.

Wikel, S. K. 1999. Tick modulation of host immunity: an important factor in pathogen transmission. Int. J. Parasitol. 29: 851–859.

Wikel, S. K., and A. C. Whelen. 1986. Ixodid-host immune interaction. Identification and characterization of relevant antigens and tick-induced host immunosuppression. Vet. Parasitol. 20: 149–174.

Wikel, S. K., R. N. Ramachandra, and D. K. Bergman. 1994. Tick-induced modulation of the host immune response. Int. J. Parasitol. 24: 59–66.