

## Article

# Surveillance of Host-Seeking Ticks in the Flint Hills Region (USA) and Associations with Environmental Determinants

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**Abstract:** Diversity and phenology of host-seeking ticks were studied in the Flint Hills region of the USA, which is a prominent region for raising beef cattle. Between a two-year period from 2015–2017, ticks were collected using the dragging method from 9 distant locations, 6 of which were studied seasonally and 3 continuously throughout the study period. Of the 10,055 ticks collected, 76.5% were the Lonestar tick (*Amblyomma americanum*), 14% were American dog tick (*Dermacentor variabilis*), and 9.1% were Gulf Coast tick (*A. maculatum*). The blacklegged tick, *Ixodes scapularis* were also found, albeit in low numbers. The host-seeking activity of all three predominant tick species peaked once during the late spring, summer months in the region. The phenology of all ticks was positively associated with accumulated temperature and photoperiod. Additionally, the Normalized Vegetation Difference Index (NDVI) was associated with *A. americanum*, and saturation deficit and relative humidity were negatively associated with *D. variabilis* and *A. maculatum* tick phenology, respectively. This finding is useful to predict the times during a year in which cattle are at higher risk for exposure to these ticks and associated pathogens.

**Keywords:** tick; *Amblyomma americanum*; *Amblyomma maculatum*; *Dermacentor variabilis*; *Ixodes scapularis*; Flint Hills; NDVI; saturation deficit; accumulated temperature; photoperiod

## 1. Introduction

Ticks are medically- and economically important hematophagous pests that affect livestock production worldwide. Estimated losses due to bovine anaplasmosis alone, which is caused by a rickettsia pathogen that is biologically transmitted by ticks ranges in the several millions of dollars every year in the US. For instance, previous estimates of the cost of a clinical bovine case due to anaplasmosis was estimated to be around \$400/animal [1,2], and in 1996 the total economic impact was conservatively estimated to be around \$300 million/year in the U.S. alone [3]. Losses due to other tick-borne diseases

to livestock and companion animals are not readily available but can be reliably expected to be in several hundreds of millions each year. Tick-borne diseases of humans and animals in the midwestern U.S. have reportedly increased in their frequency and intensity over recent years [4–10], and particularly the spatiotemporal pattern of bovine anaplasmosis in the midwestern U.S. has steadily worsened over the past decade [8].

The Flint Hills ecoregion in the Midwestern US, mostly in eastern Kansas but also in northcentral Oklahoma represents an important area for livestock production in the U.S. Poorly suited for cultivation and crop production, this area is recognized for its superior grass quality which leads to higher grazing performance by cattle. Due to the seasonality and relatively better grass quality in the Flint Hills, millions of cattle are brought to pastures from other parts of the US, which include young growing cattle (stockers) and reproductive females (i.e., cowherds), adding diversity to the complexity of the grazing population. Ticks, including *Dermacentor variabilis*, that is implicated in the transmission of *Anaplasma marginale*, the etiologic agent of bovine anaplasmosis, are commonly found in the broader region. Thus, the exposure of recently introduced naïve cattle to ticks infected by feeding on indigenous vertebrate carriers is a concern.

The present study evaluates the diversity of different questing tick species and their phenology in Flint Hills ecoregion by conducting tick surveys at 9 locations over a two-year period between 2015–2017. Tick distribution over space and time are predictable with environmental variables [11–13]. The associations of such weather conditions with tick density were evaluated using ground-level and remotely sensed data. The information generated in this study is expected to contribute to our understanding of reasons behind the current increase in bovine anaplasmosis cases in this region [8]. Thus, for improving the management of this economically important disease. Additionally, the data generated in this study will serve as a baseline for future investigations of tick diversity and phenology aimed to measure the dynamics of tick populations in this ecoregion, including those associated with ongoing biotic and abiotic (e.g., climate-change) changes in this environment.

## 2. Results

During the two-year study period from 2015–2017, a total of 10,557 ticks were collected from 9 collection sites in the Flint Hills ecoregion; 8083 (76.5%) of these ticks were identified as *Amblyomma americanum*; 1,481 (14.0%) as *D. variabilis*; 965 (9.14%) as *A. maculatum*; and 28 (0.26%) as *Ixodes scapularis*. The male/female ratio for all four species did not change during in the two years that ticks were collected, and in general, there were more females relative to males for all species in both years. The sex ratio for *A. americanum* was 0.61:1 (male: female), for *D. variabilis*, 0.69:1, and for *A. maculatum*, 0.78:1. The sex ratio for *I. scapularis* could not be reliably estimated due to the low number of samples collected. All developmental stages of *A. americanum* and adult stage *D. variabilis* were collected from all sampling sites, whereas *A. maculatum* were not collected from study sites located north of Manhattan, Kansas. *Ixodes scapularis* was collected only from two study sites in the southern part of the study region.

Among *A. americanum* ticks, larvae (33.13%) and nymphs (33.55%), all identified using PCR in pools of 10 larvae or 10 nymphs, contributed to a large portion of the collection, followed by female (20.64%) and male (12.66%) adults. Larvae and nymphs of *D. variabilis* and *A. maculatum* were not detected in the PCR analysis, i.e., not collected by flagging; the proportion of male and female ticks of these species were (41% and 59%, and 44%, 56%, respectively).

To determine the phenology of these ticks, a total of 4557 ticks were collected from the three long-term sites that were sampled each month throughout the study period. The specimens collected from these sites included, *A. americanum* males (n = 319), females (n = 523), nymphae (n = 807) and larvae (n = 969). *D. variabilis* males (n = 191) and females (n = 275); and *A. maculatum* males (n = 63) and females (n = 100) (Table 1). All the above three species of ticks were present in the three long-term sites. Regardless of the collection site, the three major tick species found in the Flint Hills ecoregion displayed a unimodal

density distribution in both years (Figure 1). Noticeable overlap in the emergence of the three post-emergent life-stages of *A. americanum* ticks was evident but the nymphs emerged relatively earlier in the season followed by adults and larvae. In both years, the peak activity of *A. americanum* nymphs occurred in May, adults peaked in June and then larvae in August. Adult density of *D. variabilis* increased in July and the emergence of *A. maculatum* adults occurred earliest in the year among the three metastriate species, in May.

**Table 1.** Tick stages collected from Flint Hills ecoregion (USA) over a two-year study period March 2015–March 2017.

Species	Year	Male	Female	Nymph	Larvae	Total
<i>Amblyomma americanum</i>	Year 1	518	782	1265	1309	3874
	Year 2	506	887	1447	1369	4209
<i>Amblyomma maculatum</i>	Year 1	222	269	-	-	491
	Year 2	203	271	-	-	474
<i>Dermacentor variabilis</i>	Year 1	321	450	-	-	771
	Year 2	286	424	-	-	710
<i>Ixodes scapularis</i>	Year 1	2	6	-	-	8
	Year 2	5	15	-	-	20

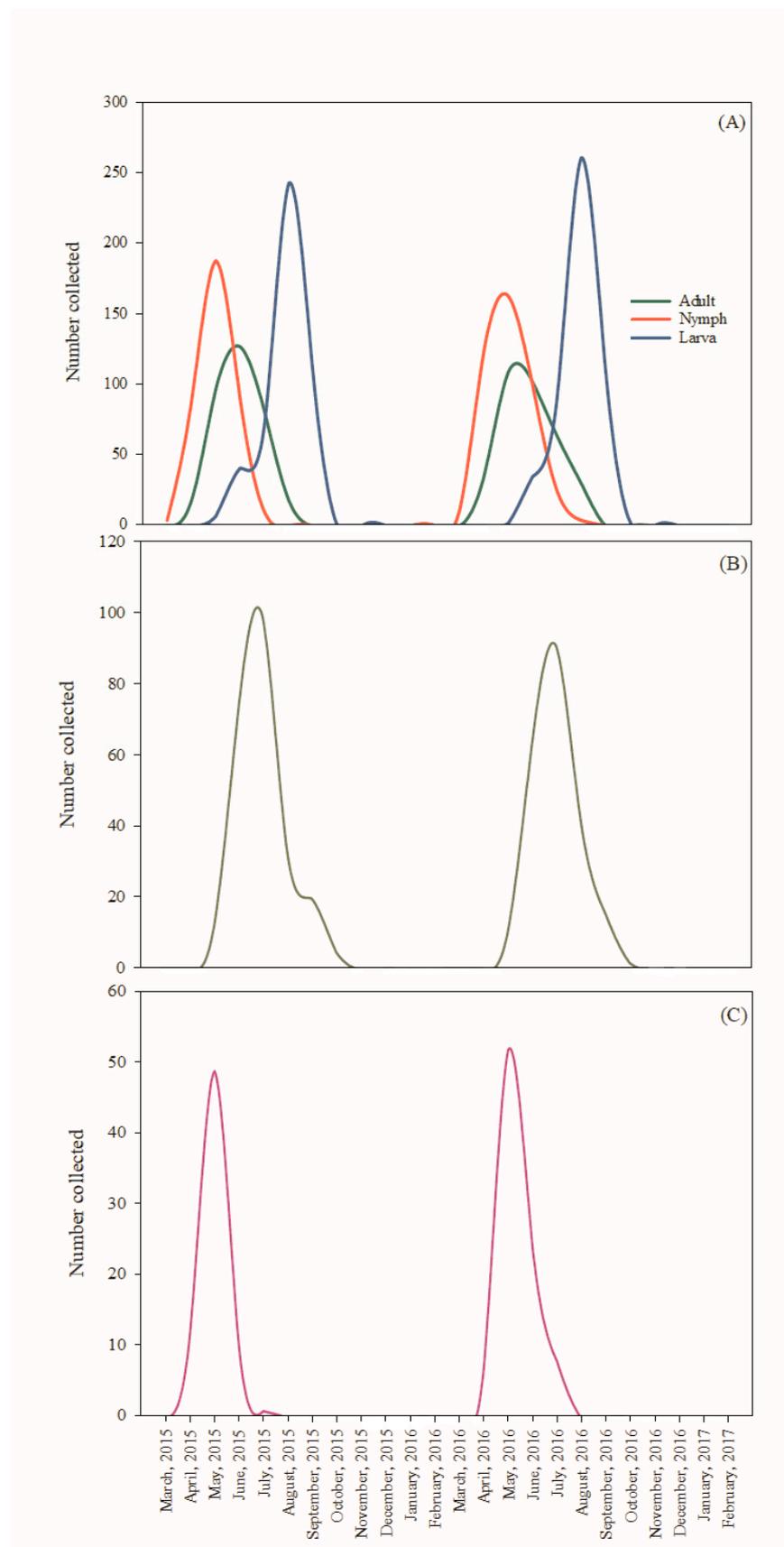
The final generalized linear mixed models (glmm) for each species evaluated climate associations with tick density revealing that accumulated temperature and photoperiod were significantly associated with all three tick species collected from the Flint Hills ecoregion between 2015–2017 (Tables 2–4). The normalized difference vegetation index (NDVI) was associated with the density of *A. americanum* ticks while saturation deficit, a measure of drying power of the air was significantly but negatively associated with *D. variabilis* (Table 4), and maximum relative humidity was negatively associated with *A. maculatum* ticks (Table 5). The random effect parameters, month of collection and location were significant in all glmm models.

**Table 2.** Associations of climate and land cover properties with density of *Amblyomma americanum* ticks (larva, nymph and adult together) fitted in a generalized linear mixed model (glmm).

Covariate	Estimate	Std. Error	Pr (>  z )	95% CI
Accumulated temperature	1.232	0.012	0.000	1.209, 1.256
Photoperiod	1.079	0.031	0.000	1.019, 1.140
NDVI	0.121	0.010	0.017	0.101, 0.1406
Location	1.553	0.496	0.015	0.580, 2.525
Month	0.811	0.213	0.007	0.039, 1.228

**Table 3.** Associations of climate variables with density of adult *Dermacentor variabilis* ticks fitted in a generalized linear mixed model (glmm).

Covariate	Estimate	Std. Error	Pr (>  z )	95% CI
Accumulated temperature	1.421	0.119	0.000	1.877, 1.654
Photoperiod	1.549	0.088	0.001	1.376, 1.721
Saturation deficit	−1.388	0.192	0.038	−1.764, −1.011
Location	1.321	0.622	0.048	0.101, 2.540
Month	0.422	0.183	0.001	0.063, 0.780



**Figure 1.** Total numbers of ticks collected every month from sampling locations in Flint Hills ecoregion (USA). (A) *Amblyomma americanum* adult, nymphae and larvae; (B) *Dermacentor variabilis* adults; (C) *Amblyomma maculatum* adults.

**Table 4.** Associations of climate variables with density of adult *Amblyomma maculatum* ticks fitted in a generalized linear mixed model (glmm).

Covariate	Estimate	Std. Error	Pr (>  z )	95% CI
Accumulated temperature	2.076	0.083	0.000	1.913, 2.238
Photoperiod	0.373	0.108	0.000	0.165, 0.588
Mean relative humidity	−0.274	0.187	0.041	−0.640, 0.092
Location	1.118	0.288	0.038	0.553, 1.682
Month	1.027	0.261	0.001	0.514, 1.538

### 3. Discussion

The objective of this study was to evaluate the diversity and phenology of questing ticks in an area where livestock production is a major economic driver, and to quantify the presence of and strength of associations between tick density and abiotic environmental drivers. To the collective knowledge of the authors, intensive tick surveys to investigate the diversity or phenology of ticks has not been conducted in the Flint Hills region in recent decades. The findings reported here therefore provide a fresh insight into the tick ecology in this region, which will be useful for designing management practices to counter the spread of bovine anaplasmosis and other tick-borne diseases. One such management practice could be the potential adjustment of the timing and/or method of burning seasonal grasses, a practice that is routinely followed by cattle ranchers in the region. Patch burning has been evaluated as a method to reduce tick burden in intensive cattle grazing areas [14,15]. These studies report a significant reduction in the number of cattle-attached ticks following patch burning and a subsequent reduction in tick-borne diseases.

The distinctive and consistent unimodal phenology of questing adults of all three tick species and the immature stages of *A. americanum*, for both years indicate that there is currently a single season during which these ticks are actively questing for bloodmeal hosts in the Flint Hills. These patterns were similar to those previously reported for *A. americanum* collected from pastured beef cattle, with overwintered adult and nymph stages appearing in the spring followed by larvae in late summer and autumn [16]. Interestingly, although it was reported that *A. americanum* nymphs and adults began questing in the Cookson Hills State Game Refuge (CHSGR) in eastern Oklahoma as early as late winter in 1973 and 1974, fed stages of these ticks were not found, in the same study, on white-tailed deer until late spring/early summer [16,17]. Thus, further work is warranted to determine if a similar phenomenon currently occurs in the Flint Hills, which are northwest of the CHSGR.

Too few *I. scapularis* ticks were collected in this study to make robust conclusions, but the adults of this species were all collected during late fall, indicating a cool-season peak for the adults and potentially its immature life-stages. Previous studies of *I. scapularis* ticks from the region indicate a similar activity period [18–20]. Tick density for the other three species appears to increase as temperatures warm-up in early spring and last until early fall; however, the peak activity for these species (and for the different life stages of *A. americanum*) occurred at different times during this roughly 6-month period, often within a narrow one- or two-month timeframe.

Accumulated temperature and photoperiod are reliable predictors of tick density in the Flint Hills, a finding similar to that reported in a recent study by Remesar et al., 2019 on *Ixodes ricinus* ticks in Spain [21]. Ticks are poikilothermic arthropods whose survival and fitness are closely linked to ambient conditions. Temperature and moisture available in the atmosphere, represented either as relative humidity or saturation deficit, have been long shown to exert significant effect on the questing behavior [22,23], development rate [24–27] and reproduction and survival [28,29] of various ixodid ticks, and they are a major limiting factor of the geographic distribution of a tick species [30–32]. NDVI, an index for remotely detecting photosynthetic activity of plants is associated with *A. americanum* density in the

present study. NDVI has been shown to correlate with the life history traits of different ticks and has been widely discussed as a reliable predictor of tick phenology in many cases [33]. The phenology of other ticks found in the Flint Hills, however, appears not to be predictable when using NDVI, as is the case with some of the other Ixodidae [21].

The density of adult *D. variabilis* ticks increased as the saturation deficit increased, indicating that the emergence and questing behavior of the adults of these ticks coincided with drier conditions in the Flint Hills. However, it is important to consider the effect of saturation deficit not in isolation but in the presence of accumulated temperature and photoperiod as these covariates together contributed to the mixed model (Table 3). The effect of photoperiod on *D. variabilis* has been shown to affect the questing behavior [26,34], with longer photoperiods increasing questing activity. Similarly, the maximum relative humidity recorded per month is associated with the density of *A. maculatum* ticks, with lower relative humidity favoring increased density.

All questing tick species collected in this study have been implicated in the transmission of pathogenic agents to humans and animals and these species are therefore intensely researched. Apart from the well-known pathogens transmitted by *A. americanum* (see: Childs and Paddock, 2002) [35], the nymphs of these ticks were recently confirmed as transmitting agents of Heartland virus (HRTV) [9,36] and Bourbon virus (BRBV) [10], which, such as rickettsial agents transmitted by these species, have been responsible for human fatalities. The intensity and frequency of other diseases transmitted by *A. americanum* have been steadily worsening over the recent years [5,7,8,37], and recently it was shown via ecological niche modeling that the potential geographic distribution of this species is broader in N. America than what was previously thought [30].

Anaplasmosis disease burden to cattle that are raised in the Flint Hills region are not accurately estimated; however, it is commonly accepted that the economic losses due to this disease are severe. It is likely that much of the reason for the prevalence of this disease in this economically vital region is caused by a combination of factors; accidental mechanical transmission of *A. marginale* during routine care of cattle and vertical transmission of the pathogen from cows to calves being some among them. American dog ticks (*D. variabilis*) have been implicated as an important biologic vector of *A. marginale*, but they are clearly not the dominant tick species in this region, accounting only 14% of the total host-seeking ticks collected in the present study. It must be noted that the immature stages of *D. variabilis* ticks in contrast with the other common ticks in this region are seldom collected using cloth drags as their questing behavior is different and closer to ground. Nevertheless, *D. variabilis* have been reported in association with cattle in nature [38,39], and this tick has been repeatedly demonstrated capable of acquisition, maintenance and transmission of *A. marginale* [40–42]. Thus, the relatively less abundant *D. variabilis* ticks play an important role as biologic vectors from subclinical carriers to naïve cattle, and possible as invertebrate maintenance and reservoir hosts of *A. marginale* during the interannual periods such that a continuous infection to naïve herds is possible [42]. The infection rate of this pathogen among *D. variabilis* ticks in Flint Hills is not presently known, and such information is warranted for evaluating the role of these ticks in the maintenance and spread of *A. marginale*.

## 4. Materials and Methods

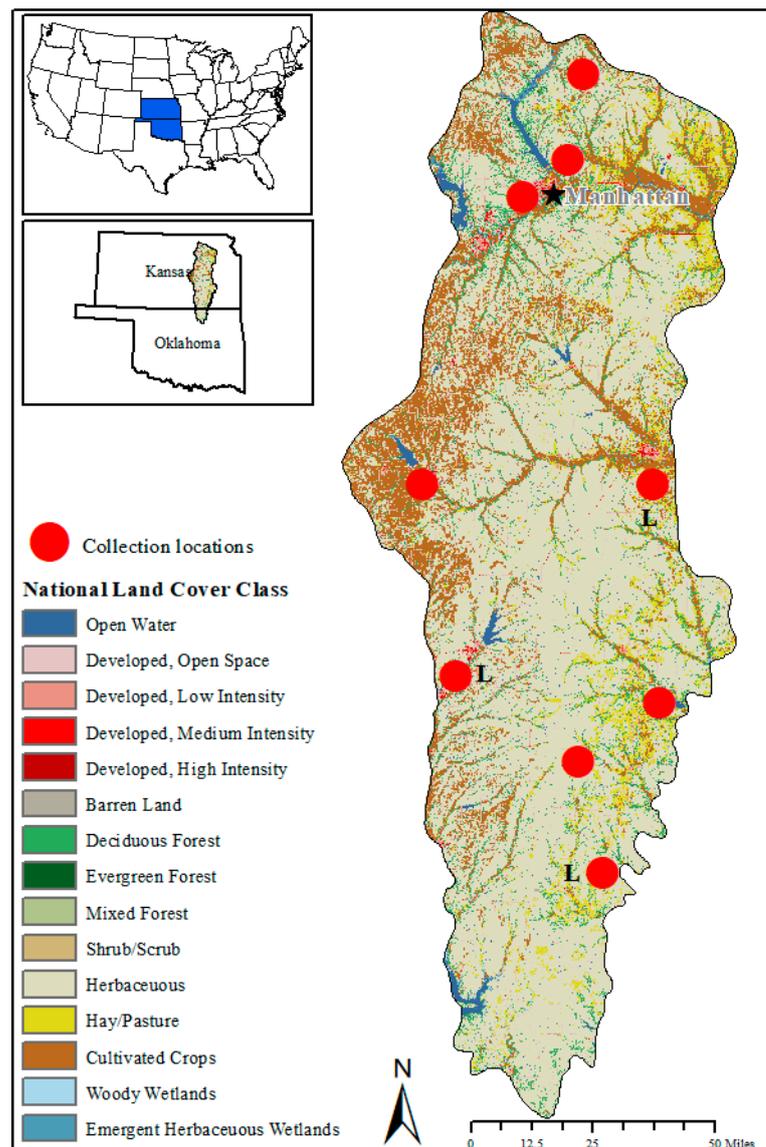
### 4.1. Study Area

This study was conducted in the Flint Hills, a narrow north-to-south region located in eastern Kansas and northcentral Oklahoma, designated by the US Environmental Protection Agency as an ecoregion characterized by dense coverage of different tallgrass species. Flint Hills landscape is mainly composed of prairie vegetation dominated by big and little bluestem, switch grass and Indian grass. Trees are seldom found, except along stream and river bottoms where they are abundant creating areas of forest-field interface. Flint Hills ecoregion covers an area of 25,333 km<sup>2</sup> with wide interannually varying continental climate. Climate conditions here range from extreme heat in summer with highs ranging between

26–43 °C, to lows in the winter dropping to −29 °C. Precipitation varies highly from year to year with average precipitation received in the area being approximately 84 cm.

#### 4.2. Tick Survey and Classification

For the evaluation of tick diversity, questing ticks were collected from 12 locations (Figure 2) between the months of March through August using a 1 m<sup>2</sup> white flannel cloth attached to a 1.3 m long flagpole for a fixed amount of time (1 h). Ticks attached to the flannel were collected every 2–5 m using forceps and stored in a plastic container until they were brought to the laboratory and placed on dry ice. Tick surveys were performed by six similarly trained biologists during the entire study period, but these tick collectors were changed once in the second year of the study.



**Figure 2.** Tick sampling sites and Flint Hills ecoregion (USA). Long-term sites are denoted by L.

To study the phenology of ticks, three sites were selected, in the north, central and southern Flint Hills ecoregion to represent any potential climate variation. From these sites, ticks were collected from 300 m<sup>2</sup> transects, once a month from the beginning of March 2015 through end of March 2017, as described above, except for periods when the transects were under snow cover and/or the temperature was below freezing. No time limit was set and entire transects were swept for ticks over the vegetation cover. Once in the lab, ticks were

placed in  $-20\text{ }^{\circ}\text{C}$  for 48–56 hours after which time they were thawed and microscopically identified to their species level using N. American tick taxonomic keys [43]. Nymphs and larvae were first identified using morphological keys, and to further confirm the species, a subset of these ticks was evaluated using molecular analysis with the PCR technique.

#### 4.3. Sample Handling and DNA Extraction

Ticks were first thawed to room temperature and identified using morphologic characteristics. For the larvae and nymphs that were not identifiable with taxonomic keys, pools of 10 larvae per pool and 10 nymphs per pool were individually prepared. Total genomic DNA from these pools were extracted using the QIAGEN DNeasy Blood and Tissue kit (QIAGEN, Valencia, CA, USA) following the recommended manufacturer's protocol with the following exceptions. The tube contents were homogenized in 2ml Lysing Matrix M screw-capped tubes filled with  $\frac{1}{4}$ " (6.35 mm) diameter of Zirconium Oxide ceramic grinding spheres (MP biomedical. LLC), and 180  $\mu\text{L}$  of ATL buffer (QIAGEN, Valencia, CA, USA), 25  $\mu\text{L}$  of proteinase K (QIAGEN, Valencia, CA, USA). These tubes were incubated at  $56\text{ }^{\circ}\text{C}$  with shaking for 1 h in a Disruptor Genie cell disruptor (Scientific Industries, Inc, Bohemia, NY, USA), and subsequently treated according to the kit instructions. DNA concentrations were measured with NanoDrop spectrophotometer (ThermoFisher, Wilmington, DE, USA) and stored at  $-20\text{ }^{\circ}\text{C}$  until used for PCR-based detection of gDNA.

##### 4.3.1. Positive Control Construction

Positive amplification controls including those for *A. americanum*, *A. maculatum* and *D. variabilis* were synthesized and cloned into a plasmid vector.

##### 4.3.2. Real-Time PCR

Oligonucleotides used for PCR are listed in Table 5 and were prepared at 10 pMol/ $\mu\text{L}$ . The singular real-time PCR assays were conducted with a Bio-Rad CFX96 Real-time PCR detection system with the iQ Multiplex Powermix kit (BioRad, Hercules, CA, USA). For each PCR, a 20  $\mu\text{L}$  reaction was prepared, which contained 10  $\mu\text{L}$  2X IQ Powermix, 5  $\mu\text{L}$  DNA template, 1  $\mu\text{L}$  probe, 1  $\mu\text{L}$  of each primer and 2  $\mu\text{L}$  nuclease free water. An initial denaturation at  $95\text{ }^{\circ}\text{C}$  for 10 min was followed by 45 cycles of  $95\text{ }^{\circ}\text{C}$  for 15 sec, the annealing and extension temperature at  $65\text{ }^{\circ}\text{C}$  for 45 s. The end results are analyzed using Bio-Rad CFX Manager 3.0 software. Annealing temperatures for individual PCR assays were first optimized using temperature gradient PCR (Bio-Rad CFX96). The optimal annealing temperatures of the assay were determined based on signal intensities of the PCR amplifications. Ct value  $\leq 30$  were considered strongly positive reactions obtained by abundant target nucleic acids in the samples. Selected amplification products were verified by electrophoresis on 1.5% agarose. The expected amplicon sizes for the respective tick species are described in Table 5

**Table 5.** Oligonucleotide primers and probes used in real-time PCR assays.

Species	Target Gene	Product Size	Primes /Probes	Sequence (5'-3')
<i>Amblyomma americanum</i>	16S rRNA	150bp	* FP * RP * Pr	TTTAATTGGGGCGATTTAACTA CATCGAGGTCGCAAACACTATT FAM-GAACCGTTATTAACGGACACTTGGA-BHQ-1
<i>Amblyomma maculatum</i>	16S rRNA	178bp	* FP * RP * Pr	AAGGACAAGAAGACCCTAAGAATTT ATTACGCTGTTATCCCTAGAGTATT CAL Fluor Red 610-TGAAATTTTTTAATTGGGGCGA-BHQ-2
<i>Dermacentor variabilis</i>	16S rRNA	138bp	*FP * RP * Pr	TGGTATTTTGACTATACAAAGGTATT CCTTAATTTAATAATTGTTTCTTCAC CAL Fluor Gold 540- TGCTAAGAGAATGGAATTACAGGGAATA-BHQ-1

\* FP: Forward primer; RP: Reverse primer; Pr: Probe.

#### 4.4. Environmental Data

Climate and landcover properties affect tick biology [44]. Daily climate data for the study period, closest to the sampling locations were obtained from the National Weather Service (NWS) and cooperative stations. This data included temperature (minimum, maximum and mean), relative humidity, precipitation and wind speed. Daily evapotranspiration and soil moisture index were derived from the raw climate records. Vegetation index data for this analysis was extracted from the MOD13A2 product derived from the MODIS instrument onboard the EOS-Terra platform. Vegetation indices from this product (NDVI, EVI) are calculated from the blue, red and near infrared MODIS bands using daily atmosphere-corrected bidirectional reflectance values over 16-day compositing periods. Composite index values for the 16-day periods are selected to represent each compositing period by culling all pixels whose quality assurance metrics fall below a minimum standard, then selecting the highest index value from the view angle that is closest to nadir. The study site was located on the H10V05 MODIS tile.

#### 4.5. Statistical Analysis

The association between climate and land cover covariates with tick density for each species was evaluated in a Generalized Linear Mixed Model construct using the glmm package (see Knudson, 2018) [45] in R-Statistical program [46]. The correlation among covariates were first assessed and pairs of covariates with R value >8 were removed. Model over-fitting can lead to finding erroneous associations. Therefore, before fitting the glmm model, the strength of associations between tick density and different fixed-effect covariates were individually screened by fitting univariate models with a liberal cut-off *P*-value. Only those fixed-effect covariates that retained statistical significance ( $p \leq 0.1$ ) were kept for the glmm model; in which, log normalized tick density was the dependent variable, different climate and land cover covariates were fixed-effect independent variables, and location, month and year of tick collection were kept as random-effect variables. A full model with all screened variables and random effects was fitted first, followed by several mixed models with fewer fixed-effect covariates at each step without those that were non-significant ( $p \leq 0.05$ ). These steps were followed independently for each tick species.

## 5. Conclusions

This study evaluated the diversity of different questing tick species and their phenology in the Flint Hills ecoregion and the authors describe the presence of three major tick species having similar density distribution with overlap in emergence. Specifically, the increase in adult density of *D. variabilis* in late July will be a helpful guide for understanding regional and within-herd risk for transmission of *A. marginale*, the primary etiologic agent of bovine anaplasmosis. The information described in this study also provides a foundation for future studies on the monitoring of tick population dynamics in this important ecoregion of the Midwest. Further research is warranted in the year-to-year variation in the emergence of the tick as well as the ability of the ticks within a region to harbor the disease agents without continued reinfection from reservoir cattle.

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