Seasonal life cycle of the Lone Star Tick (*Amblyomma americanum*) and the influence of prescribed burning on tick abundance and presence of microorganisms

by

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B.S.E., Armed Forces University – ESPE, 2016
M.S. Oklahoma State University, 2020

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Entomology
College of Agriculture

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Abstract

The lone star tick (*Amblyomma americanum*) is the most common and widely distributed tick species in the Central and Eastern United States and dominates tick communities in the Kansas Flint Hills. All life stages of *A. americanum* feed on humans as well as a variety of vertebrate hosts, serving as vectors for several pathogens, such as *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Francisella tularensis*, and Heartland virus. In addition to pathogen transmission, infestations of this tick can cause irritation, blood loss, and reduced productivity in livestock. This dissertation examined the ecology of *A. americanum* in the Kansas Flint Hills and evaluated prescribed burning as a long-term, sustainable method to manage tick populations and reduce tick-borne pathogen burdens. The research was conducted at the Kansas State University Beef Stocker Unit, a tallgrass prairie site where prescribed burning was applied in different seasons (spring, summer, and fall). Understanding how prescribed fire affects tick abundance, seasonal activity, and pathogen presence is integral to developing integrated management practices that protect human and animal health.

In Chapter 2, the seasonal life cycle of *A. americanum* populations under field conditions in Kansas was studied. Field collections suggest that the life cycle takes two years to complete, which was similar to reports from other regions in the Central United States. In year one, adults lay eggs in early summer, and larvae emerge from late July through October. Fed larvae or newly molted nymphs overwinter, with nymphs reappearing as early as March in year two. Nymph ticks remain active through summer, molting into adults by late summer or fall.

In Chapter 3, I evaluated the effects of long-term prescribed burning on *A. americanum* populations. The results showed a significant reduction in tick abundance in burned pastures compared to the unburned control. Burns performed in the spring were seen to be the most

effective. Consecutive applications of prescribed burning over several years kept a low tick population, which indicates a lasting suppressive effect of the fire.

In Chapter 4, the impact of prescribed burning on the presence of microorganisms in field-collected *A. americanum was investigated*. Overall, 36.7% of ticks were positive for *Rickettsia* spp., 8.3% for *E. chaffeensis*, and 5.3% for *E. ewingii*, while *R. rickettsii* and *F. tularensis* were not detected. Although prescribed burning significantly reduced tick abundance, it did not affect the presence of tested microorganisms across treatments. Co-occurrence analysis showed that *E. chaffeensis* and *E. ewingii* were detected together more often than expected by chance, suggesting possible interactions within the tick or during feeding; the two species were acquired from the same host. In general, this research contributes to a better understanding of *A. americanum* ecology, its response to prescribed fire, and pathogen dynamics in the Flint Hills region.

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Chapter 1 - Literature review and Dissertation structure

Abstract

Ticks are important ectoparasites of humans and animals. They transmit a variety of pathogens and can also harm their hosts directly through blood loss, skin irritation, and secondary infections. In some cases, tick bites can cause paralysis or allergic reactions, such as alpha-gal syndrome. In the United States, *Amblyomma americanum* (the lone star tick) is widely distributed across the Eastern United States, ranging from Central Texas, Oklahoma, and Kansas to the Atlantic coast as far north as Maine. In Kansas, this tick species has been found across several eastern counties, including the Kansas Flint Hills, which is the largest intact tallgrass prairie in the world. This region provides high-quality forage that supports excellent cattle growth. All stages of *A. americanum* can feed on humans, domestic animals, and wildlife. This broad host range increases the potential transmission of pathogens, such as *Ehrlichia chaffeensis, Ehrlichia ewingii*, and Heartland virus. Traditional tick management depends on acaricides, but chemical control can be expensive, environmentally damaging, and may lead to resistance over time. Due to these limitations, there is a growing interest in alternative control methods, such as biological control, vegetation management, and prescribed fire. Prescribed burning, which is already used in the Kansas Flint Hills to improve forage quality, may also help to reduce tick populations. However, its effectiveness varies depending on frequency, intensity, seasonality, and severity of the fire. This dissertation examined the ecology of *A. americanum*, the presence of tick-borne pathogens, and the role of prescribed burning as a management strategy in the Kansas Flint Hills. In Chapter 2, I described the seasonal life cycle of *A. americanum* in a warm, cattle-grazed tallgrass prairie ecosystem. In Chapter 3, I assessed whether sustained prescribed burning impacts tick abundance. In Chapter 4, I assessed the influence of prescribed burning on the presence of tick-borne

pathogens and endosymbionts in *A. americanum*. Overall, this research demonstrated the importance of understanding tick ecology, seasonal dynamics, the presence of microorganisms, and prescribed burning as a potential strategy to reduce tick populations and tick-borne pathogen transmission.

Introduction

Ticks (Acari: Ixodida) are ectoparasites that feed on the blood of terrestrial and semi-aquatic vertebrates (Guglielmone et al. 2014). They belong to the phylum Arthropoda, class Arachnida, and order Acari (Barker and Murrell 2004). Worldwide, around 900 tick species have been described and are grouped into three families: *Ixodidae* (hard ticks), *Argasidae* (soft ticks), and *Nuttalliellidae* (one species, *Nuttalliella namaqua*) (Nava et al. 2009, Guglielmone et al. 2010, Thomas and Reichard 2021). Of the three families, *Ixodidae* is the most important because its members play an important role in the transmission of pathogens that affect both humans and animals (Perumalsamy et al. 2024).

Hard ticks are divided into two groups based on their morphology: Prostriata and Metastriata (Brites-Neto et al. 2015). Prostriata species are recognized by a prominent anterior anal groove and include only one subfamily, Ixodinae, which contains the genus, *Ixodes* (Brites-Neto et al. 2015, Perumalsamy et al. 2024). In contrast, Metastriata species have a small posterior slit-like anal groove and are divided into four subfamilies: *Amblyomminae*, with the genera *Amblyomma* and *Aponomma*; *Haemaphysalinae* with the genus *Haemaphysalis*; *Hyalomminae* with the genus *Hyalomma*; and *Riphicephalinae* composed of seven genera: *Dermacentor, Cosmiomma, Margaropus, Nosomma, Anomalohimalaya, Rhipicentor,* and *Rhipicephalus* (Brites-Neto et al. 2015, Perumalsamy et al. 2024).

Morphology of ticks

The body of hard ticks is divided into three regions: the gnathosoma or capitulum, the main body known as the idiosoma, and segmented legs (Figure 1) (Sonenshine and Roe 2014). The gnathosoma contains mouthparts, which include palps, chelicerae, hypostome, and basis capituli (Sonenshine and Roe 2014). The idiosoma houses the internal organs, such as the salivary glands, midgut, Malpighian tubules, and reproductive structures. The legs, composed of multiple segments, are attached to the coxae on the anterior portion of the idiosoma. At the distal end of the forelegs lies Haller's organ, a specialized sensory structure that detects odors, temperature, and humidity from the environment (Sonenshine and Roe 2014, Thomas and Reichard 2021). Morphological features of ticks are used to distinguish between species.

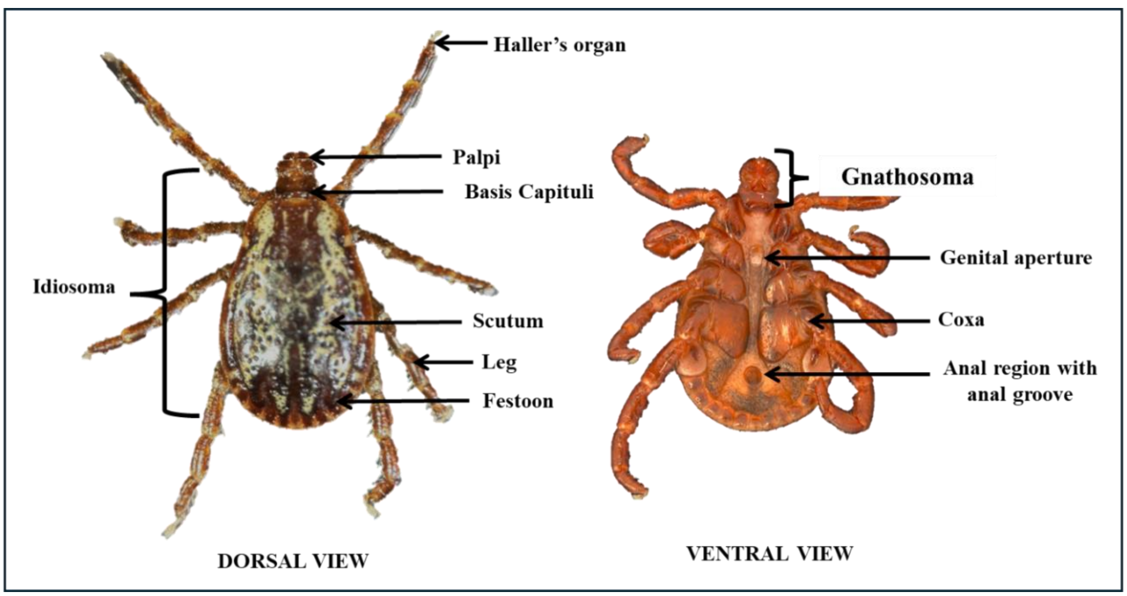


Figure 1.1. Morphological characteristics of a hard tick (family *Ixodidae*), showing *Dermacentor variabilis* male in dorsal and ventral views.

Life cycle of ticks

The life cycle of hard ticks consists of four developmental stages: eggs, larvae, nymphs, and adults. After hatching, each motile stage requires host-seeking, blood-feeding, metamorphosis, and a period of dormancy before moving to the next stage. Reproduction occurs during the adult stage (Thomas and Reichard 2021, Perumalsamy et al. 2024). *Amblyomma* and *Dermacentor* female ticks reach full engorgement only after mating, as the male transfers an engorgement factor to the female during copulation (Weiss and Kaufman 2004). Depending on the species, ticks may follow a one-host, two-host, or three-host life cycle. The three-host pattern is the most common, with larvae and nymphs feeding on small animals and adults preferring larger vertebrates, depending on host availability and species-specific behavior (Thomas and Reichard 2021, Perumalsamy et al. 2024). The life cycle is determined by the number of hosts a tick feeds on over its life (Figure 2).

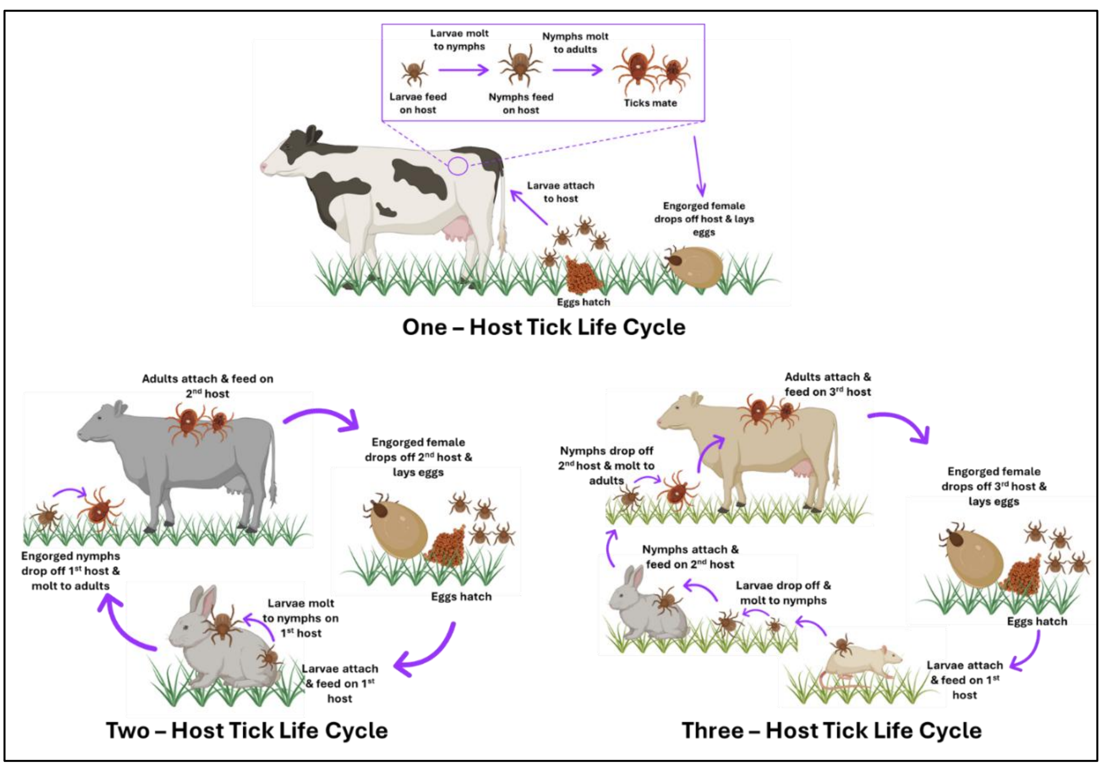


Figure 1.2. Life cycles of hard ticks.

All hard ticks must take a blood meal during each stage of their life cycle. Host-seeking behavior differs among tick genera and often occurs in two forms: questing and hunting. *Rhipicephalus, Haemaphysalis*, and *Ixodes* species use questing behavior, climbing onto vegetation, and extending their forelegs where the Haller's organ is located to detect and attach to passing hosts (Perumalsamy et al. 2024, Marshall et al. 2025). In contrast, *Amblyomma* and *Hyalomma* have questing and hunting behaviors. They move across the ground to search for their hosts to detect cues like host-emitted carbon dioxide (Perumalsamy et al. 2024, Marshall et al. 2025).

Global importance of ticks

Worldwide, ticks are second only to mosquitoes as the most important arthropod vectors of pathogens affecting humans and animals (Jongejan and Uilenberg 2004, Dantas-Torres et al. 2012). This concern continues to grow with the increase in tick-borne disease cases. The Centers for Disease Control and Prevention (CDC) has reported more than 89,000 cases of Lyme disease, caused by the *Borrelia burgdorferi,* in 2023. Other zoonotic diseases transmitted by ticks, such as babesiosis, ehrlichiosis, and anaplasmosis, are expanding into new geographical regions, which increases attention from physicians and veterinarians. In addition, *Theileria orientalis* Ikeda, the causative agent of bovine theileriosis, has recently caused large and severe outbreaks in the United States (Iduu et al. 2023, Ierardi et al. 2025).

In the United States, several hard tick species are particularly important to human and animal health. These include *Amblyomma americanum* (the lone star tick), *A. maculatum* (the Gulf Coast tick), *Dermacentor andersoni (*the Rocky Mountain wood tick), *D. occidentalis* (the Pacific coast tick), *D. variabilis* (the American dog tick), *Ixodes pacificus* (the western blacklegged tick), *I. scapularis* (the blacklegged tick or deer tick), *Rhipicephalus sanguineus* sensu lato (the brown dog tick), and *Haemaphysalis longicornis* (Asian longhorned tick). A summary of these species, the pathogens they transmit, and the diseases associated with them is shown in Table 1.

Table 1.1. Tick-borne diseases in the United States, associated pathogens, tick vector species, hosts, and geographic distribution (Sonenshine and Roe 2014, Eisen et al. 2017, Perumalsamy et al. 2024).

Disease	Pathogen	Tick species	Main host	Geographic Distribution
Lyme disease	*Borrelia burgdorferi*	*Ixodes scapularis*, *Ixodes pacificus*	Rodents (reservoirs), deer, humans	Northeast, Upper Midwest, Pacific Coast
Human Anaplasmosis	*Anaplasma phagocytophilum*	*Ixodes scapularis*, *Ixodes pacificus*,	Rodents, deer, humans	Northeast, Midwest, Pacific Coast
Bovine Anaplasmosis	*Anaplasma marginale*	*Dermacentor variabilis*, *D. andersoni*, *Rhipicephalus sanguineus*	Cattle (primary), wild ruminants	Endemic in Southern and Western U.S.; outbreaks in Southeast
Babesiosis	*Babesia microti* (humans), *B. vogeli*, *B. gibsoni* (dogs)	*Ixodes scapularis* *Rhipicephalus sanguineus*	Rodents, dogs, humans, cattle	Northeast, Upper Midwest
Bovine babesiosis	*Babesia bovis* *Babesia bigemina*	*Rhipicephalus microplus* *Rhipicephalus annulatus*	Cattle	Southern U.S.
Ehrlichiosis	*Ehrlichia chaffeensis*, *E. ewingii*, *E. canis*	*Amblyomma americanum*	Deer, dogs, humans	Southeastern, South-central U.S.

		Rhipicephalus sanguineus		
Bovine Theileriosis	*Theileria orientalis* (Ikeda genotype)	*Haemaphysalis longicornis*	Cattle (primary), deer	Detected in Virginia and expanding in the Eastern U.S.
Rocky Mountain spotted fever (RMSF)	*Rickettsia rickettsii*	*Dermacentor variabilis*, *D. andersoni*, *Rhipicephalus sanguineus*	Dogs, rodents, humans	Eastern U.S., Rocky Mountains, Southwest
Rickettsia parkeri **rickettsiosis**	*Rickettsia parkeri*	*Amblyomma maculatum*	Deer, dogs, humans	Gulf Coast, California, Oregon
Pacific Coast tick fever	*Rickettsia philipii*	*Dermacentor occidentalis*	Deer, dogs, humans	Gulf Coast, California, Oregon
Tularemia	*Francisella tularensis*	*Amblyomma americanum*, *Dermacentor variabilis*, *D. andersoni*	Rabbits, rodents, humans	Nationwide; highest risk in central U.S.
Heartland virus disease	Heartland virus	*Amblyomma americanum*	Small mammals, deer, humans	Midwestern and Southeastern U.S.
Bourbon virus disease	Bourbon virus	*Amblyomma americanum*	Small mammals, deer, humans	Midwest U.S.
Colorado tick fever	Colorado tick fever virus	*Dermacentor andersoni*	Rodent, deer, humans	Rocky Mountain region (Western U.S.)

Powassan encephalitis	Powassan virus	*Ixodes scapularis*	Rodents (reservoirs), humans	Northeastern and Upper Midwestern U.S.
Other conditions	Alpha-gal syndrome (meat allergy)	*Amblyomma americanum*	Humans	Southeastern & South-central U.S.

Larvae, nymphs, and adult ticks can transmit infectious agents to their host, but how they facilitate transmission depends on the pathogen. For example, transovarial or vertical transmission, also known as vertical transmission, occurs when larvae acquire pathogens passed from an infected female tick to the developing eggs (Kleinjan and Lane 2008). More commonly, pathogens are acquired via a transstadial transmission, in which ticks become infected while feeding on a host that carries the pathogen (Kleinjan and Lane 2008). The pathogen survives through molting into the next life stage and is later transmitted horizontally to a new host during the next blood meal (Perumalsamy et al. 2024).

Direct damage caused by ticks

In addition to transmitting various pathogens, ticks can also cause direct harm to their hosts. During feeding, they consume significant amounts of blood, which in cases of heavy infestations can result in anemia, weight loss, toxicosis, and reduced productivity in livestock (Gerem Eskezia and Hadush Desta 2016, Basu and Charles 2017). Tick bites also cause irritation, inflammation, and skin damage, which can make the host more susceptible to secondary bacterial infections (Abubakar and Perera 2019). Some ticks, such as *Ixodes* and *Dermacentor*, are capable of inducing tick paralysis in their host through neurotoxins released in their saliva (Basu and Charles 2017). In humans, tick bites can trigger severe allergic reactions, including anaphylaxis or delayed hypersensitivity responses, such as alpha-gal syndrome (Perumalsamy et al. 2024).

Tick-borne pathogens are responsible for high levels of morbidity and mortality in animals and contribute to economic losses worldwide. In the United States, livestock infestations cause significant losses, and the United States Department of Agriculture (USDA) has estimated that a large-scale cattle tick (*R. microplus*) outbreak could cost more than $1.2 billion, just in control measures (McClung et al. 2023). These impacts underscore the need for effective tick surveillance and management strategies to protect both public health and livestock production.

Tick species commonly found in Kansas

In Kansas, the main tick species include *Amblyomma americanum* (lone star tick), *Dermacentor variabilis* (American dog tick), *Dermacentor albipictus* (winter tick), *Ixodes scapularis* (blacklegged tick or deer tick), *Rhipicephalus sanguineus* (brown dog tick), and *Haemaphysalis leporispalustris* (rabbit tick) (Peterson 2012, Ng'eno et al. 2024, Olds 2025). A survey conducted across the Central Great Plains of Kansas and Oklahoma found that 97% of the ticks collected were *A. americanum,* followed by *D. variabilis* (2%)*, D. albipictus* (1%), and less than 1% *I. scapularis* and *A. maculatum* (Ng'eno et al. 2024). Seasonal differences in tick activity also showed that *A. americanum, A. maculatum,* and *D. variabilis* were most abundant in spring and summer, and *D. albipictus* and *I. scapularis* were primarily active during fall and winter (Ng'eno et al. 2024).

Amblyomma americanum

Amblyomma americanum (Linnaeus, 1758), commonly known as the lone star tick, is widely distributed across the Eastern United States, extending from Central Texas, Oklahoma, and Kansas, to the Atlantic coast as far north as Maine (Figure 3) (CDC 2019, Raghavan et al. 2019). This species follows a three-host tick and exhibits aggressive and non-specific feeding behavior, parasitizing a wide range of hosts, including humans and animals (Goddard and Varela-Stokes

2009). Larvae and nymphs commonly feed on both small and large mammals as well as ground-feeding birds, while adults primarily parasitize medium to large mammals (Goddard and Varela-Stokes 2009). All three life stages feed on humans and are capable of transmitting tick-borne pathogens (Goddard and Varela-Stokes 2009).

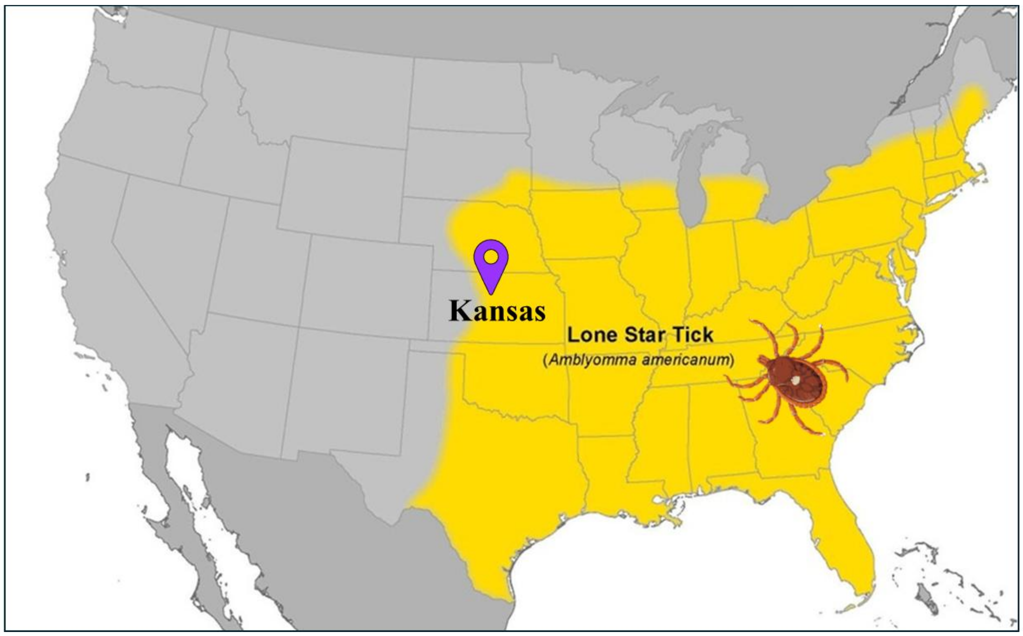


Figure 1.3. Distribution of *Amblyomma americanum* across the United States, with Kansas indicated as part of its range (CDC 2019).

The seasonal activity of *A. americanum* extends from early spring through fall (Goddard 2007, Goddard and Varela-Stokes 2009). In warmer climates, adults may begin to emerge as early as February, reaching the highest numbers in May or June before declining by late July. Nymphs, which overwinter as unfed flat nymphs, become active in March and may remain active through August or September, often showing two peaks of activity (one in late spring and another in late summer). Larvae are usually active from June to October, with the highest numbers observed in August.

Environmental factors such as temperature and humidity influence the length of the tick life cycle (Bouzek et al. 2013). Under laboratory conditions, ticks can complete development in less than 22 weeks if feeding occurs soon after molting. However, the typical cycle requires 28-30 weeks (Troughton and Levin 2007). In the field, the life cycle often extends over multiple years due to periods of inactivity, especially in adults (Bouzek et al. 2013). Research conducted in northeast Missouri found that the life cycle usually takes at least two years to complete, which provides a useful reference point for comparisons with populations in other regions, such as Kansas (Bouzek et al. 2013) (Figure 4).

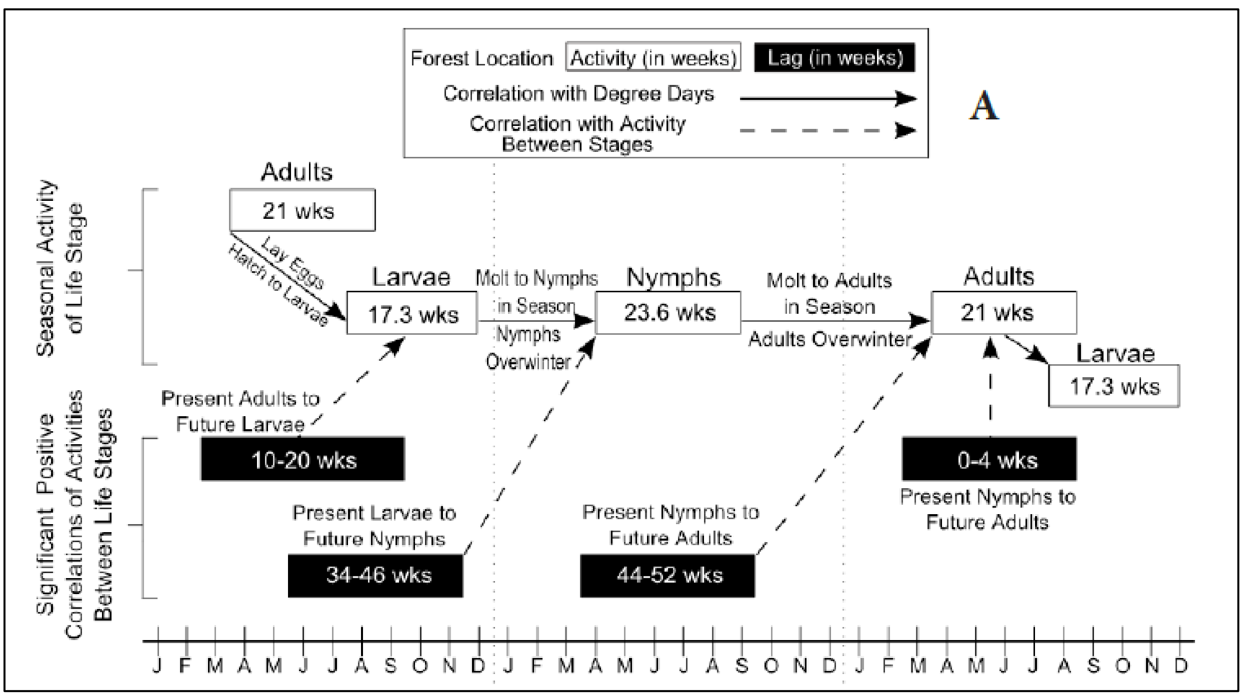


Figure 1.4. Schematic illustration of the *Amblyomma americanum* life cycle from observations in northeast Missouri (Bouzek et al. 2013).

As an aggressive host seeking species, *A. americanum* plays a major role in the transmission of pathogens that pose risks to public health. This species has been linked to the transmission of pathogens that cause diseases such as ehrlichiosis, tularemia, and Southern tick-associated rash illness (STARI) (Childs and Paddock 2003, Petersen and Schriefer 2005, Masters et al. 2008, Goddard and Varela-Stokes 2009). In addition to these diseases, tick bites have been associated with alpha-gal syndrome, which is a delayed allergic reaction to red meat caused by IgE antibodies against the oligosaccharide galactose-α-1,3-galactose (Commins et al. 2011, Crispell et al. 2019). Wounds produced by tick mouthparts may also serve as an initiation point for a New World screwworm infestation (Alexander 2006). A summary of the pathogens associated with this species is presented in Table 2. Because of its broad distribution, diverse host range, and ability to transmit multiple pathogens across all life stages, *A. americanum* represents a serious risk to human and animal health. Understanding tick ecology, vector-host associations, and tick distribution is essential for improving tick control strategies, preventing disease transmission, and supporting public health initiatives.

Table 1.2. Summary of pathogens and diseases transmitted by *Amblyomma americanum* (Goddard and Varela-Stokes 2009).

Pathogen	Disease	Hosts	Detection Source	Reference
Ehrlichia chaffeensis	Human monocytic ehrlichiosis	Humans, dogs, cattle, white-tailed deer	Field-collected ticks (widely detected)	(Cohen et al. 2010)
Ehrlichia ewingii	Human *E. ewingii* granulocytic ehrlichiosis	Humans, dogs, white-tailed deer	Field-collected ticks (common)	(Cohen et al. 2010)
Rickettsia rickettsii	Rocky Mountain spotted fever	Humans, dogs, rodents (reservoirs)	Rare in *A. americanum* (mainly lab-confirmed transmission)	(Sayler et al. 2016)
Rickettsia amblyommatis	Spotted fever group (mild disease)	Humans	Field-collected ticks (very frequent endosymbiont)	(Yen et al. 2021)

Rickettsia parkeri	Spotted fever group rickettsiae	Humans	Not typically found in *A. americanum* (mainly lab-confirmed transmission)	(Goddard 2003)
Francisella tularensis	Tularemia	Humans, dogs, etc.	Occasionally field-detected in *A. americanum*, but not a primary vector	(Goddard & Varela-Stokes 2009)
Possibly Borrelia lonestari	STARI	Humans, white-tailed deer	Field-collected ticks (pathogenicity uncertain)	(Varela-Stoke 2007)
Heartland virus	Heartland virus disease	Humans, small animals	Field-collected ticks	(Egizi et al. 2023)
Bourbon virus	Bourbon virus disease	Humans, small animals	Field-collected ticks	(Egizi et al. 2023)

Amblyomma americanum also carries a variety of microbial endosymbionts that are mainly found in the salivary glands, Malpighian tubules, and reproductive organs (Maldonado-Ruiz et al. 2021, Kumar et al. 2022). Microbiome studies have identified non-pathogenic bacteria such as *Coxiella*-like endosymbionts and *Rickettsia amblyommatis* in this species (Klyachko et al. 2007, Karpathy et al. 2016). Although *R. amblyommatis* was only considered an endosymbiont, some studies reported that it may also behave as a pathogen, suggesting a dual role in tick biology and disease transmission (Billeter et al. 2007). Endosymbionts can influence tick physiology by contributing to nutritional, reproduction, and immune function. They may also interact with tick-borne pathogens in ways that either promote or inhibit pathogen development within the vector (Hussain et al. 2022). For example, a reduction of *Coxiella*-like endosymbionts in *R. haemaphysaloides* facilitated the colonization of *Babesia microti,* whereas the presence of *R. amblyommatis* in *A. americanum* was associated with lowering the transstadial transmission rate of *Rickettsia rickettsii* (Levin et al. 2018, Li et al. 2018, Zhong et al. 2024). Because both pathogens and endosymbionts can affect tick physiology, vectorial capacity, and host health, accurate detection and characterization of these microorganisms are essential for understanding tick ecology and disease dynamics, and for developing effective strategies for tick and pathogen control.

Tick Control Methods

As tick distribution continues to expand, the development of effective management strategies has become important to mitigate the problems they cause. Several approaches have been used to control tick populations on hosts and in the environment, like chemical control, biological control, pasture management, anti-tick vaccines, and the use of tick-resistant cattle breeds (Basu and Charles 2017, Perumalsamy et al. 2024).

Chemical controls

Acaricides are the most common control methods used to kill ticks. These can be plant extracts, organochlorines, organophosphates, carbamates, formamidines, synthetic pyrethroids, and macrocyclic lactones (Rajput et al. 2006, Perumalsamy et al. 2024). Acaricides are typically applied to animals during periods of tick activity by dipping, spraying, ear tags, collars, pour-on formulations, or baths, to reduce the large number of ticks feeding on the host (Basu and Charles 2017). Despite their effectiveness, chemical control presents several drawbacks, including residues in meat or milk products, the emergence of resistant tick strains, toxicity to mammals, and negative impacts on the environment and non-target organisms (Polito et al. 2013, Rochlin and Toledo 2020).

Applying acaricides across large herds or over extensive pastures requires substantial financial investment, making this approach impractical for many producers. Furthermore, this approach only affects ticks when they are feeding on the hosts and does not target off-host stages, such as ticks feeding on wildlife, molting, laying eggs, or overwintering in the environment

(Perumalsamy et al. 2024). Consequently, a significant portion of the tick life cycle remains unaffected. These limitations have increased interest in non-chemical control options, including biological control, vegetation management, and the use of tick-tolerant animal breeds.

Biological controls

Biological control uses the natural enemies of the pest, such as pathogens, parasitoids, and predators, to help reduce tick populations (Perumalsamy et al. 2024). Ants, beetles, spiders, mites, parasitoid wasps, nematodes, and microbial pathogens can be employed to decrease tick populations (Dantas-Torres et al. 2012, Basu and Charles 2017). However, this type of control has not been widely adopted for tick management due to limitations related to its effectiveness, practicality, cost, and the complexity of tick biology.

Vegetation management

Two and three-host ticks spend most of their lives off the host in the vegetation. During these off-host periods, they are exposed to various environmental conditions, including heat, drying, and cold weather. Modification of vegetation by mowing grass, removing leaf litter, and clearing dense trees can reduce the humid microclimates that ticks depend on and limit their opportunities to find a host and obtain a blood meal (Eisen and Stafford III 2021). Mowing has proven to be one of the most effective management strategies for controlling *H. longicornis* populations. As this thesis focuses on controlling ticks on grazing pasture, it will only address prescribed burning.

Prescribed burning

Prescribed burning has been proposed as a non-chemical alternative for managing tick populations, especially in grazing systems. While other vegetation management practices, such as mowing and clearing, can reduce tick habitat, fire also changes the microclimates, host abundance, and vegetation structure in ways that can directly and indirectly affect tick numbers. Previous studies have reported conflicting results on the effectiveness of this method. Some have found no significant changes in tick abundance, while others have shown clear reductions in populations immediately after burning and up to a year after the treatment was applied (Davidson et al. 1994, Padgett et al. 2009, Willis et al. 2012, Polito et al. 2013, Gleim et al. 2014). These discrepancies often depend on burn frequency, intensity, seasonality, and characteristics of the area (Gleim et al. 2014). Therefore, further research is needed to identify the specific conditions under which prescribed fire is most effective in reducing tick populations.

In the Midwest, prescribed burning is a common land management practice used to improve forage quality, suppress invasive weeds, soil nutrient cycling, and maintain the biodiversity of tallgrass prairies (Bloomer et al. 2024). Fire can also affect small-animal populations and distributions. Evaluating its potential as a tool for tick control is important, given the growing incidence of tick-borne diseases and the limitations of chemical acaricides. To better understand its role, studies should consider ecological factors, such as host availability, vegetation type, and landscape use, that influence tick survival and host-seeking behavior.

The impact of prescribed fire on tick populations depends on fire characteristics, such as frequency, intensity, seasonality, and severity (Scasta et al. 2023). Frequent burns tend to lower tick abundance by continually removing leaf litter and understory vegetation, which reduces suitable habitats for ticks. In contrast, fire treatments, which are applied once in several years, do not have a long-term effect on tick populations (Davidson et al. 1994, Cully 1999, Fill et al. 2023). High intensity burns consume deeper litter layers and lower soil moisture, which can directly kill ticks or decrease their survival during winter (Ferrenberg et al. 2006, Allan 2009, Scasta 2015, Gallagher et al. 2022). The reductions in vegetation cover and litter layers also create hotter and drier habitats, increasing desiccation and lowering molting success (Ogden et al. 2004, Alexander et al. 2021, Gallagher et al. 2022). The timing of burns within the year has not been addressed and may vary across ecological zones and tick species. In general, prescribed fire represents a promising option for integrated tick management. The success of prescribed fire depends on the ecosystem and characteristics of the fire regime. Continued research is needed to determine the most effective way to implement this practice, balancing the reduction of tick populations with the ecological benefits of fire in grazing systems.

Cattle production in the Kansas Flint Hills

Kansas is the third-largest cattle-producing state in the U.S., with 5.95 million cattle on ranches and feedyards as of 2025, according to the Kansas Livestock Association. In 2023, the cattle and calves industry generated around $13.7 billion in cash receipts (Kansas Ag Statistics, 2023). Ranching is the leading agricultural activity in Kansas, especially in the Flint Hills region, which forms the largest portion of the U.S. stocker cattle industry (Malin 1942, Duncan 2020). The Flint Hills is a native tallgrass prairie known for its high productivity and good quality forage, which allows cattle to gain up to three pounds per day under ideal conditions (Ott 2016).

Each year, more than one million yearling cattle from across the U.S. are brought to the Flint Hills for the summer grazing season, which is from April to September (Duesterhaus et al. 2008, Beam 2010, Towne and Craine 2016). Ranchers in this region commonly use continuous grazing, where cattle stay in the same pasture for the season, which is economically efficient and sustainable (Owensby and Fick 2016). Prescribed burning is routinely applied before the grazing season to improve forage growth and livestock performance (Bernardo et al. 1988, Towne and Craine 2016). Because of the economic and ecological value of the Kansas Flint Hills, the presence of ticks has become a growing concern for livestock health and productivity. This highlights the need for effective control methods to protect productivity in this region.

Dissertation structure and objectives

In this dissertation, I studied the seasonal abundance of *A. americanum* life stages, the presence of tick-associated microorganisms, and the effects of prescribed burning as a possible approach to reduce tick populations and pathogen transmission. This research was conducted at the Kansas State University Beef Stocker Unit to model general conditions across the Flint Hills region.

In Chapter 2, I examined the life cycle of *A. americanum* in the Kansas Flint Hills. A previous study from northeast Missouri reported that this species requires approximately two years to complete its life cycle. Using that information as a reference, ticks were collected over a three-year period to identify periods of tick activity that may increase the risk of tick bites and tick-borne pathogens in humans and animals. The findings from this part of the research emphasize the importance of ongoing monitoring and the application of effective tick control methods.

In Chapter 3, I evaluated the potential of long-term prescribed burning as a method to mitigate tick populations. Prescribed burning has been used in the Kansas Flint Hills since the late nineteenth century to improve forage quality and cattle performance. At the Beef Stocker Unit, a consistent prescribed burning program has been maintained for the past five years. Ticks were collected from pastures with different burn treatments to determine how fire timing and frequency affect tick abundance and whether seasonality plays a role in the efficacy of fire.

In Chapter 4, I tested the hypothesis that prescribed burning influences the presence of microorganisms in *A. americanum* collected from the field. Screening was performed to identify pathogenic and non-pathogenic microorganisms associated with this tick species and to determine if the prescribed fire reduces the presence of microorganisms in ticks collected from burned areas.

References

Abubakar M, Perera PK. 2019. *Ticks and Tick-Borne Pathogens, BoD–Books on Demand.*

Alexander HD, Siegert C, Brewer JS et al. 2021. Mesophication of oak landscapes: Evidence, knowledge gaps, and future research. BioScience 71(5):531-542. https://doi.org/10.1093/biosci/biaa169.

Alexander JL. 2006. Screwworms. Journal of the American Veterinary Medical Association 228(3):357-367. https://doi.org/10.2460/javma.228.3.357.

Allan BF. 2009. Influence of prescribed burns on the abundance of *Amblyomma americanum* (Acari: *Ixodidae*) in the Missouri Ozarks. Journal of Medical Entomology 46(5):1030-1036. https://doi.org/10.1603/033.046.0509.

Barker S, Murrell A. 2004. Systematics and evolution of ticks with a list of valid genus and species names. Parasitology 129(S1):S15-S36. https://doi.org/10.1017/S0031182004005207.

Basu AK, Charles R. 2017. Ticks of Trinidad and Tobago: an overview.

Beam M. 2010. Beef production in the Flint Hills. https://doi.org/newprairiepress.org/sfh/2010/nature/5.

Bernardo D, Engle D, McCollum E. 1988. An economic assessment of risk and returns from prescribed burning on tallgrass prairie. Rangeland Ecology & Management/Journal of Range Management Archives 41(2):178-183. https://doi.org/10.2458/azu_j_rang_v41i2_178_183.

Billeter SA, Blanton HL, Little SE et al. 2007. Detection of "*Rickettsia amblyommii*" in association with a tick bite rash. Vector-Borne and Zoonotic Diseases 7(4):607-610. https://doi.org/10.1089/vbz.2007.0121.

Bloomer CC, Miller CM, DiStefano RJ et al. 2024. Midwest prairie management practices benefit the non-target prairie crayfish. Fire Ecology 20(1):11. https://doi.org/10.1186/s42408-023-00243-x.

Bouzek D, Foré S, Bevell J et al. 2013. A conceptual model of the *Amblyomma americanum* life cycle in northeast Missouri. Journal of Vector Ecology 38(1):74-81. https://doi.org/10.1111/j.1948-7134.2013.12011.x.

Brites-Neto J, Duarte KMR, Martins TF. 2015. Tick-borne infections in human and animal population worldwide. Veterinary World 8(3):301. https://doi.org/10.14202/vetworld.2015.301-315.

CDC. 2019. Geographic distribution of ticks that bite humans. https://doi.org/www.cdc.gov/ticks/about/where-ticks-live.html.

Childs JE, Paddock CD. 2003. The ascendancy of *Amblyomma americanum* as a vector of pathogens affecting humans in the United States. Annual review of entomology 48(1):307-337. https://doi.org/10.1146/annurev.ento.48.091801.112728.

Cohen SB, Yabsley MJ, Freye JD et al. 2010. Prevalence of *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in ticks from Tennessee. Vector-Borne and Zoonotic Diseases 10(5):435-440. https://doi.org/10.1089/vbz.2009.0058.

Commins SP, James HR, Kelly LA et al. 2011. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-α-1, 3-galactose. Journal of

Allergy and Clinical Immunology 127(5):1286-1293. e1286. https://doi.org/10.1016/j.jaci.2011.02.019.

Crispell G, Commins SP, Archer-Hartman SA et al. 2019. Discovery of alpha-gal-containing antigens in North American tick species believed to induce red meat allergy. Frontiers in immunology 10:1056. https://doi.org/10.3389/fimmu.2019.01056.

Cully JF. 1999. Lone star tick abundance, fire, and bison grazing in tallgrass prairie. Rangeland Ecology & Management/Journal of Range Management Archives 52(2):139-144. https://doi.org/10.2307/4003507.

Dantas-Torres F, Chomel BB, Otranto D. 2012. Ticks and tick-borne diseases: a One Health perspective. Trends in parasitology 28(10):437-446. https://doi.org/10.1016/j.pt.2012.07.003.

Davidson WR, Siefken DA, Creekmore LH. 1994. Influence of annual and biennial prescribed burning during March on the abundance of *Amblyomma americanum* (Acari: *Ixodidae*) in central Georgia. Journal of Medical Entomology 31(1):72-81. https://doi.org/10.1093/jmedent/31.1.72.

Duesterhaus J, Ham J, Owensby C et al. 2008. Water balance of a stock-watering pond in the Flint Hills of Kansas. Rangeland Ecology & Management 61(3):329-338. https://doi.org/10.2111/07-097.1.

Duncan ZM. 2020. Effects of prescribed fire timing on yearling stocker cattle performance, native plant composition, forage biomass accumulation, and root carbohydrate concentrations in key native tallgrass plant species. https://doi.org/10.1093/tas/txab077.

Egizi A, Wagner NE, Jordan RA et al. 2023. Lone star ticks (Acari: *Ixodidae*) infected with Bourbon virus in New Jersey, USA. Journal of Medical Entomology. https://doi.org/10.1093/jme/tjad052.

Eisen L, Stafford III KC. 2021. Barriers to effective tick management and tick-bite prevention in the United States (Acari: *Ixodidae*). Journal of Medical Entomology 58(4):1588-1600. https://doi.org/10.1093/jme/tjaa079.

Eisen RJ, Kugeler KJ, Eisen L et al. 2017. Tick-borne zoonoses in the United States: persistent and emerging threats to human health. ILAR journal 58(3):319-335. https://doi.org/10.1093/ilar/ilx005.

Ferrenberg SM, Schwilk DW, Knapp EE et al. 2006. Fire decreases arthropod abundance but increases diversity: early and late season prescribed fire effects in a Sierra Nevada mixed-conifer forest. Fire Ecology 2:79-102. https://doi.org/10.4996/fireecology.0202079.

Fill JM, Miller HM, Crandall R. 2023. Prescribed Fire as a Tool for Controlling Tick Populations in the Southeastern United States: FOR398/FR469, 8/2023. EDIS 2023(4). https://doi.org/10.32473/edis-fr469-2023.

Gallagher MR, Kreye JK, Machtinger ET et al. 2022. Can restoration of fire-dependent ecosystems reduce ticks and tick-borne disease prevalence in the eastern United States? Ecological Applications 32(7):e2637. https://doi.org/10.1002/eap.2637.

Gerem Eskezia B, Hadush Desta A. 2016. Review on the impact of ticks on livestock health and productivity. J. Bio. Agr. Health 6:1-7.

Gleim ER, Conner LM, Berghaus RD et al. 2014. The phenology of ticks and the effects of long-term prescribed burning on tick population dynamics in southwestern Georgia and northwestern Florida. PLoS One 9(11):e112174. https://doi.org/10.1371/journal.pone.0112174.

Goddard J. 2003. Experimental infection of lone star ticks, *Amblyomma americanum* (L.), with *Rickettsia parkeri* and exposure of guinea pigs to the agent. Journal of Medical Entomology 40(5):686-689. https://doi.org/10.1603/0022-2585-40.5.686.

Goddard J. 2007. Seasonal activity of *Amblyomma* spp. in Mississippi. Journal of Vector Ecology 32(1):157-158. https://doi.org/10.3376/1081-1710(2007)32[157:SAOASI]2.0.CO;2.

Goddard J, Varela-Stokes AS. 2009. Role of the lone star tick, *Amblyomma americanum* (L.), in human and animal diseases. Veterinary parasitology 160(1-2):1-12. https://doi.org/10.1016/j.vetpar.2008.10.089.

Guglielmone AA, Robbins RG, Apanaskevich DA et al. 2014. The hard ticks of the world. 10. https://doi.org/10.1007/978-94-007-7497-1.

Guglielmone AA, Robbins RG, Apanaskevich DA et al. 2010. The *Argasidae, Ixodidae* and *Nuttalliellidae* (Acari: Ixodida) of the world: a list of valid species names. https://doi.org/10.5281/zenodo.196488.

Hussain S, Perveen N, Hussain A et al. 2022. The symbiotic continuum within ticks: Opportunities for disease control. Frontiers in Microbiology 13:854803. https://doi.org/10.3389/fmicb.2022.854803.

Iduu N, Barua S, Falkenberg S et al. 2023. *Theileria orientalis* Ikeda in cattle, Alabama, USA. Veterinary Sciences 10(11):638. https://doi.org/10.3390/vetsci10110638.

Ierardi RA, Chance SM, Morris C et al. 2025. Active surveillance for *Theileria orientalis* and the invasive Asian longhorned tick (*Haemaphysalis longicornis*) in three Missouri beef herds. Plos one 20(4):e0319327. https://doi.org/10.1371/journal.pone.0319327.

Jongejan F, Uilenberg G. 2004. The global importance of ticks. Parasitology 129(S1):S3-S14. https://doi.org/10.1017/s0031182004005967.

Karpathy SE, Slater KS, Goldsmith CS et al. 2016. *Rickettsia amblyommatis* sp. nov., a spotted fever group *Rickettsia* associated with multiple species of *Amblyomma* ticks in North, Central, and South America. International journal of systematic and evolutionary microbiology 66(12):5236. https://doi.org/10.1099/ijsem.0.001502.

Kleinjan JE, Lane RS. 2008. Larval keys to the genera of *Ixodidae* (Acari) and species of *Ixodes* (Latreille) ticks established in California. The Pan-Pacific Entomologist 84(2):121. https://doi.org/10.3956/2007-38.1.

Klyachko O, Stein BD, Grindle N et al. 2007. Localization and visualization of a *Coxiella*-type symbiont within the lone star tick, *Amblyomma americanum*. Applied and environmental microbiology 73(20):6584-6594. https://doi.org/10.1128/AEM.00537-07.

Kumar D, Sharma SR, Adegoke A et al. 2022. Recently evolved *Francisella*-like endosymbiont outcompetes an ancient and evolutionarily associated *Coxiella*-like endosymbiont in the lone star tick (*Amblyomma americanum*) linked to the Alpha-Gal Syndrome. Frontiers in Cellular and Infection Microbiology 12:425. https://doi.org/10.3389/fcimb.2022.787209.

Levin ML, Schumacher LB, Snellgrove A. 2018. Effects of *Rickettsia amblyommatis* infection on the vector competence of *Amblyomma americanum* ticks for *Rickettsia rickettsii*. Vector-Borne and Zoonotic Diseases 18(11):579-587. https://doi.org/10.1089/vbz.2018.2284.

Li L-H, Zhang Y, Zhu D et al. 2018. Endosymbionts alter larva-to-nymph transstadial transmission of *Babesia microti* in *Rhipicephalus haemaphysaloides* ticks. Frontiers in microbiology 9:1415. https://doi.org/10.3389/fmicb.2018.01415.

Maldonado-Ruiz LP, Neupane S, Park Y et al. 2021. The bacterial community of the lone star tick (*Amblyomma americanum*). Parasites & Vectors 14:1-10. https://doi.org/10.1186/s13071-020-04550-z.

Malin JC. 1942. An introduction to the history of the bluestem-pasture region of Kansas. Kansas Historical Quarterly 11(1):3-28. https://doi.org/kancoll.org/khq/1942/42_1_malin.htm.

Marshall DS, Poh KC, Reichard MV et al. 2025. Spatial and temporal activity patterns of *Amblyomma americanum.* Parasites & Vectors 18(1):12. https://doi.org/10.1186/s13071-025-06661-x.

Masters EJ, Grigery CN, Masters RW. 2008. STARI, or Masters disease: Lone Star tick–vectored Lyme-like illness. Infectious disease clinics of North America 22(2):361-376. https://doi.org/10.1016/j.idc.2007.12.010.

McClung KL, Sundstrom KD, Lineberry MW et al. 2023. Seasonality of *Amblyomma americanum* (Acari: *Ixodidae*) activity and prevalence of infection with tick-borne disease agents in north central Oklahoma. Vector-Borne and Zoonotic Diseases 23(11):561-567. https://doi.org/10.1089/vbz.2023.0009.

Nava S, Guglielmone AA, Mangold AJ. 2009. An overview of systematics and evolution of ticks. https://doi.org/10.2741/3418.

Ng'eno E, Alkishe A, Romero-Alvarez D et al. 2024. Phenology of five tick species in the central Great Plains. Plos one 19(5):e0302689. https://doi.org/10.1371/journal.pone.0302689.

Ogden N, Lindsay L, Beauchamp G et al. 2004. Investigation of relationships between temperature and developmental rates of tick *Ixodes scapularis* (Acari: *Ixodidae*) in the laboratory and field. Journal of Medical Entomology 41(4):622-633. https://doi.org/10.1603/0022-2585-41.4.622.

Olds C. 2025. Ticks in Kansas. https://doi.org/bookstore.ksre.ksu.edu/pubs/ticks-in-kansas_MF2653.

Ott HL. 2016. Economic analysis of backgrounding and stocking industries in the Flint Hills of Kansas. https://doi.org/krex.k-state.edu.

Owensby C, Fick W. 2016. Summer grazing strategies for stocker cattle in the Kansas Flint Hills. MF (Kansas State University, Agricultural Experiment Station and Cooperative Extension Service).

Padgett K, Casher L, Stephens S et al. 2009. Effect of prescribed fire for tick control in California chaparral. Journal of Medical Entomology 46(5):1138-1145. https://doi.org/10.1603/033.046.0522.

Perumalsamy N, Sharma R, Subramanian M et al. 2024. Hard ticks as vectors: the emerging threat of tick-borne diseases in India. Pathogens 13(7):556. https://doi.org/10.3390/pathogens13070556.

Petersen J, Schriefer M. 2005. Tularemia: emergence/re-emergence. Veterinary research 36(3):455-467. https://doi.org/10.1051/vetres:2005006.

Peterson JR. 2012. Distribution, seasonal occurrence, abundance, and hosts of five Kansas ticks (Acari: *Ixodidae*).

Polito VJ, Baum KA, Payton ME et al. 2013. Tick abundance and levels of infestation on cattle in response to patch burning. Rangeland ecology & management 66(5):545-552. https://doi.org/10.2111/REM-D-12-00172.1.

Raghavan RK, Peterson AT, Cobos ME et al. 2019. Current and future distribution of the lone star tick, *Amblyomma americanum* (L.)(Acari: *Ixodidae*) in North America. PloS one 14(1):e0209082. https://doi.org/10.1371/journal.pone.0209082.

Rajput ZI, Hu S-h, Chen W-j et al. 2006. Importance of ticks and their chemical and immunological control in livestock. Journal of Zhejiang University Science B 7(11):912-921. https://doi.org/10.1631/jzus.2006.B0912.

Rochlin I, Toledo A. 2020. Emerging tick-borne pathogens of public health importance: a mini-review. Journal of Medical Microbiology 69(6):781. https://doi.org/10.1099/jmm.0.001206.

Sayler KA, Loftis AD, Beatty SK et al. 2016. Prevalence of tick-borne pathogens in host-seeking *Amblyomma americanum* (Acari: *Ixodidae*) and *Odocoileus virginianus* (Artiodactyla: *Cervidae*) in Florida. Journal of Medical Entomology 53(4):949-956. https://doi.org/10.1093/jme/tjw054.

Scasta JD. 2015. Fire and parasites: an under-recognized form of anthropogenic land use change and mechanism of disease exposure. EcoHealth 12:398-403. https://doi.org/10.1007/s10393-015-1024-5.

Scasta JD, Twidwell D, Donovan V et al. 2023. Role and management of fire in rangelands. Rangeland wildlife ecology and conservation:147-175. https://doi.org/10.1007/978-3-031-34037-6_6.

Sonenshine DE, Roe RM. 2014. *Biology of ticks, volume 2, Oxford University Press.*

Thomas JE, Reichard MV. 2021. Ticks'. *Elsevier. 1359-1377.*

Towne EG, Craine JM. 2016. A critical examination of the timing of burning in the Kansas Flint Hills. Rangeland Ecology & Management 69(1):28-34. https://doi.org/10.1016/j.rama.2015.10.008.

Troughton DR, Levin ML. 2007. Life cycles of seven ixodid tick species (Acari: *Ixodidae*) under standardized laboratory conditions. Journal of Medical Entomology 44(5):732-740. https://doi.org/10.1603/0022-2585(2007)44[732:lcosit]2.0.co;2.

Varela-Stokes AS. 2007. Transmission of bacterial agents from lone star ticks to white-tailed deer. Journal of Medical Entomology 44(3):478-483. https://doi.org/10.1093/jmedent/44.3.478.

Weiss BL, Kaufman WR. 2004. Two feeding-induced proteins from the male gonad trigger engorgement of the female tick *Amblyomma hebraeum*. Proceedings of the National Academy of Sciences 101(16):5874-5879. https://doi.org/10.1073/pnas.0307529101.

Willis D, Carter R, Murdock C et al. 2012. Relationship between habitat type, fire frequency, and *Amblyomma americanum* populations in east-central Alabama. Journal of Vector Ecology 37(2):373-381. https://doi.org/10.1111/j.1948-7134.2012.00241.x.

Yen W-Y, Stern K, Mishra S et al. 2021. Virulence potential of *Rickettsia amblyommatis* for spotted fever pathogenesis in mice. Pathogens and Disease 79(5):ftab024. https://doi.org/10.1093/femspd/ftab024.

Zhong Z, Wang K, Wang J. 2024. Tick symbiosis. Current Opinion in Insect Science:101163. https://doi.org/10.1016/j.cois.2024.101163.

Chapter 2 - *Amblyomma americanum* life cycle in Kansas Flint Hills

Abstract

The lone star tick, *Amblyomma americanum*, is the dominant tick species in the south-central United States and serves as an important vector of pathogens that affect both humans and livestock. While its general life cycle has been well described, less is known about how seasonal activity varies across regions, especially in the tallgrass prairie environment. This study investigated the seasonal dynamics of *A. americanum* life stages in the Kansas Flint Hills. Ticks were collected biweekly over three consecutive years (2023-2025) using drag cloth and dry-ice traps. The total number of ticks collected was analyzed over time to determine the abundance of adults, nymphs, and larvae in relation to minimum and maximum temperatures and relative humidity. Results showed a consistent sequence of life-stage activity throughout the study period. Adults were most active in early spring under cool and moist conditions (minimum temperatures ≥10 °C, relative humidity 60–80%), nymphs peaked in late spring and summer when temperatures were moderate and humidity remained above 65%, and larvae was most abundant in late summer, with reaching the highest numbers in August under warm temperatures (~28–30 °C) and high humidity (~70%). Although the overall seasonal pattern remained similar from year to year, the duration of tick activity varied. In 2023, wetter spring conditions coincided with higher numbers of nymphs and larvae, whereas in 2024, drier conditions were associated with lower abundance and a longer but weaker activity period. These results suggest that temperature determines when ticks become active, while humidity has a stronger influence on how long and how intensely populations persist. Understanding these seasonal patterns is important for predicting how *A. americanum* populations respond to climate shifts and for improving or implementing tick control

strategies, such as scheduling prescribed burning that coincides with tick phenology in the Kansas Flint Hills.

Introduction

The lone star tick (*Amblyomma americanum*) is a three-host species known for its aggressive and non-selective feeding behavior across all stages (Bouzek et al. 2013, Ludwig et al. 2016). It is a vector of a variety of pathogens that affect humans and animals, including *Ehrlichia chaffeensis, Ehrlichia ewingii, Francisella tularensis,* spotted fever group *Rickettsia*, and the causative agent of southern tick-associated rash illness (STARI) (Childs and Paddock 2003, Stromdahl and Hickling 2012, Ma et al. 2021). This tick species has also been associated with the transmission of viral agents such as Heartland virus and the development of alpha-gal syndrome, which is a red meat allergy in humans (Commins et al. 2011, Savage et al. 2016).

Amblyomma americanum is widely distributed throughout the Southeastern and Eastern United States, with a significant presence in Kansas (Raghavan et al. 2016, Raghavan et al. 2019). In recent years, its range has continued to expand into northern states because of climate change (Ma et al. 2021). As a three-host tick, it spends most of its life off the host, which makes it highly susceptible to environmental conditions such as temperature, humidity, and precipitation (Raghavan et al. 2016, Ma et al. 2021). Temperature plays an important role in the developmental process of this tick. Cooler temperatures tend to slow the molting and increase the mortality rate, while warmer conditions speed up the development and reduce the duration of the tick life cycle, resulting in higher population growth (Raghavan et al. 2016, Sagurova et al. 2019, Ma et al. 2021). Additionally, ticks are highly vulnerable to desiccation during their off-host periods, so adequate humidity allows them to survive and successfully colonize new areas (Raghavan et al. 2016).

In most geographic areas, *A. americanum* typically completes its life cycle in approximately two years, although this period can vary depending on temperature, humidity, and day length of the region where ticks are developing, as well as when ticks get a blood meal (Bouzek et al. 2013, Ma et al. 2021). In the southern regions with warmer climates, such as Texas and Mississippi, model simulations suggest that the whole tick life cycle may be completed in approximately 99 weeks (Haile and Mount 1987). Field studies from Oklahoma and Missouri have found that the life cycle spans approximately two years (Bouzek et al. 2013, Cobos et al. 2024). In these areas, adults and nymphs often emerge in early spring and remain active through late summer, while larval activity begins later in summer and declines by late fall (Bouzek et al. 2013). In contrast, in cooler northern areas, including parts of the northern United States and southern Canada, the extended winters can prolong the tick development to more than two years, with some ticks requiring up to three full seasons to complete the cycle (Sagurova et al. 2019).

The Flint Hills region of Kansas forms the largest remaining area of tallgrass prairie in North America (Knapp et al. 1998). Within this landscape, *A. americanum* is the most abundant and active tick species (Ng'eno et al. 2024). Variations in climate and vegetation structure across this region can influence tick activity patterns, altering the timing and abundance of each life stage throughout the year. Identifying when adults, nymphs, and larvae are most active is necessary for developing tick prevention strategies, including habitat management, optimal times for acaricide application, and personal protective measures. Together, these approaches can help to reduce the risk of tick-borne pathogen transmission and enhance public health and livestock productivity. To address this need, the present study analyzed multi-year field data to describe the seasonal patterns of *A. americanum* life stages in the Kansas Flint Hills.

Materials and Methods

Sampling site

This study was conducted in the Kansas State University Beef Stocker Unit located west of Manhattan, Kansas (39°13′48.80″N, 96°38′35.56″W). The site covers approximately 450 hectares of native tallgrass prairie dominated by warm-season grasses, such as big bluestem, little bluestem, Indiangrass, and sideoats grama. Although the unit has historically burned pasture, we focused on an area where prescribed burning has not been applied for at least five years. Within this section, three permanent sampling locations were established: Area 1 (39°14′21.49″N, 96°38′13.58″W), Area 2 (39°14′21.49″N, 96°38′13.58″W), and Area 3 (39°13′48.80″N, 96°38′35.56″W) (Figure 2.1). Each sampling area contained both a tree-line section and an open grass section to minimize vegetation-related variation in tick collections.

Figure 2.1. Kansas State University Stocker Unit area with marker pins showing the specific sites where *Amblyomma americanum* sampling was conducted.

Tick collection methods and identification

Ticks were collected biweekly from the three sampling areas between February and November during 2023, 2024, and 2025. All life stages of *A. americanum* were sampled using a combination of drag cloth and dry ice baiting methods (Koch and McNew 1981, Zimmerman et al. 1987, Barré et al. 1997, Petry et al. 2010). For drag sampling, a 100 × 100 cm flannel cloth was attached to a 120 cm wooden stick (2.5 cm diameter) along one edge, with each end of the stick tied to about 150 cm of twine. This setup allowed the cloth to be pulled close to the ground across a 20 m^2 area in a single pass. Ticks adhering to the cloth were carefully removed with forceps and placed into sterile 50 ml centrifuge tubes. Following this, approximately 2.5 pounds of dry ice were placed on a plastic plate set on a 100 × 100 cm flannel cloth and left to sublimate for about one hour in a shaded area near the tree line.

Ticks collected from both sampling methods were stored at -20 °C for 24 hours to ensure mortality before processing. In the laboratory, specimens of *A. americanum* were identified by life stage and sex and then counted under a stereomicroscope. The taxonomic identification was performed based on morphological characteristics, following published taxonomic keys (Keirans and Litwak 1989, Durden and Beati 2013, Dubie et al. 2017).

Data analysis

The normality of the data was assessed using the Shapiro-Wilk test. Since the data did not meet the assumption of normality ($p < 0.05$), non-parametric methods were used. Differences among the three areas were evaluated with the Kruskal-Wallis test for each year (2023, 2024, and 2025) and for each tick life stage (larvae, nymphs, and adults). When significant differences were detected, Dunn's multiple comparisons test was applied to identify pairwise differences. Statistical significance was set at $p < 0.05$. All analyses were performed in GraphPad Prism (GraphPad Software, San Diego, CA, USA).

Seasonal activity patterns of *A. americanum* were examined by plotting biweekly tick counts from 2023 to 2025. For each sampling date, tick counts from the three sampling areas and both collection methods (drag cloth and dry ice baiting) were combined. Total counts for each life stage (larvae, nymphs, and adults) were visualized using time series plots to show seasonal patterns and year-to-year variation. Bar graphs were created with overlaid trend lines showing the minimum and maximum temperatures (°C) and relative humidity (%) to explore how the abundance related to environmental conditions. Environmental data were obtained from the Kansas Mesonet website (https://mesonet.k-state.edu) using records from the Manhattan weather station. All graphs were generated in RStudio (version 4.5.1).

Results

Seasonal dynamics of *Amblyomma americanum*

A Kruskal-Wallis test showed no significant differences in adult tick counts among the three sites in 2023 ($H = 1.189$, $df = 2$, $p = 0.552$), in 2024 ($H = 2.932$, $df = 2$, $p = 0.231$), and in 2025 ($H = 3.394$, $df = 2$, $p = 0.1832$). However, nymph counts varied significantly among sites in 2023 ($H = 6.896$, $df = 2$, $p = 0.032$). Dunn's multiple comparisons indicated that Area 2 had significantly higher nymphs than Area 1 ($p = 0.026$), while Area 3 did not differ from either ($p > 0.05$). In 2024, differences in nymphs were not statistically significant ($H = 5.126$, $df = 2$, $p = 0.077$). By 2025, nymph counts varied significantly among sites ($H = 13.41$, $df = 2$, $p = 0.001$), with Area 3 showing higher nymphal abundance than both Area 1 ($p = 0.007$) and Area 2 ($p = 0.003$), while no difference was observed between Area 1 and Area 2 ($p > 0.999$).

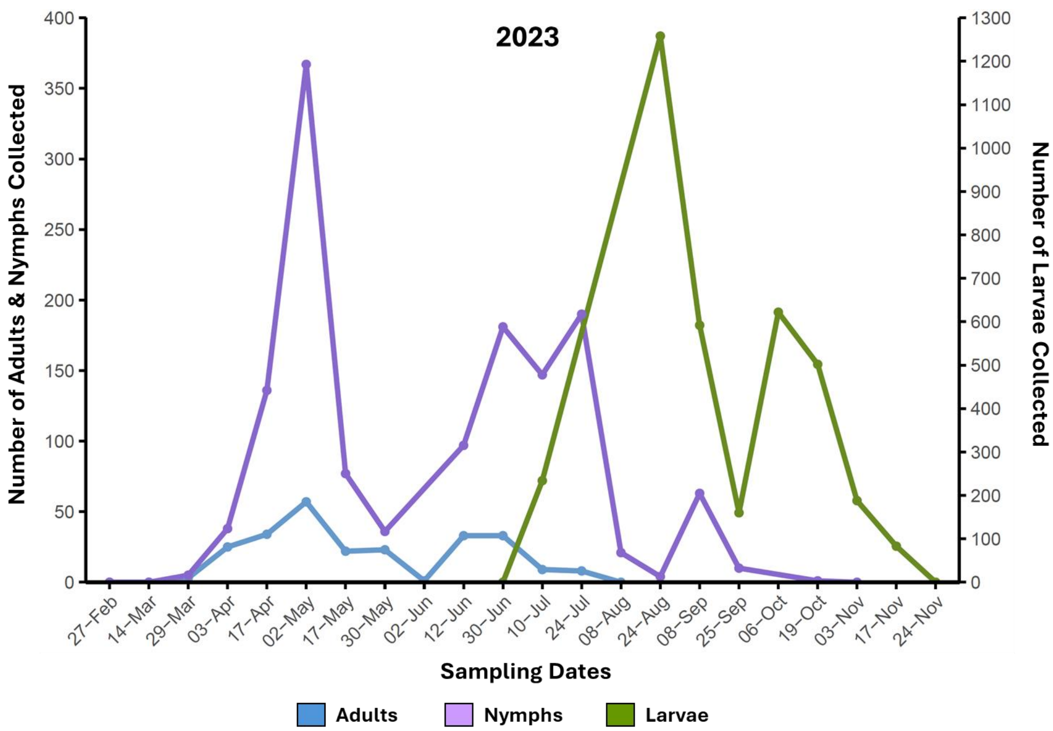
For larvae, sampling site differences were not significant in 2023 ($H = 2.588$, $df = 2$, $p = 0.274$). In 2024, larval abundance differed significantly among sites ($H = 7.613$, $df = 2$, $p = 0.022$), and Dunn's test showed that Area 2 had more larvae than Area 3 ($p = 0.021$), whereas Area 1 did not differ from either ($p > 0.05$). In 2025, collected larval populations again did not vary significantly among sites ($H = 0.426$, $df = 2$, $p = 0.864$).

Across all the years, *A. americanum* displayed a consistent seasonal cycle typical of populations in Kansas, although the timing and magnitude of activity of each stage varied from year to year (Figure 2.2).

Adults (blue line, Figure 2.2) were the first life stage to appear each season, becoming active as early as late February in 2025 and by mid-March 2023 and 2024. Adult peaks occurred in spring, with the highest counts in early May 2023 (over 60 ticks) and late March 2024 (over 150 ticks). In 2025, adults reached their maximum abundance in mid-April (around 70 ticks). The activity of adult ticks declined rapidly by June, with only a few collected during the summer months.

Nymphs (purple line, Figure 2.2) were the most abundant and widely distributed life stage throughout the year. Their activity patterns differed over sampling years. In 2023, nymphs exhibited a sharp peak in early May (over 350 ticks), followed by fluctuating numbers through July and August and another increase in mid-summer and early September. In 2024, nymph population peaked earlier, around late March, but with lower abundance (around 90 ticks). Despite this, nymphs remained active until September, indicating a more prolonged season but less intense activity period. In 2025, nymphs peaked again in mid-April (over 110 ticks), followed by steady levels through early summer and a smaller rise in July to August. Nymphs continued their activity until October, with only a few remaining at the end of the month.

Larvae (green line, Figure 2.2) were the last stage to emerge, typically appearing in July and reaching the highest numbers in late summer. The largest larval peak was observed in August 2023, when counts exceeded 1,200 ticks. In 2024, overall larval abundance was lower but showed multiple peaks in July and August, suggesting an extended hatching and development period. In 2025, the number of larvae increased in early August (over 1,100 ticks), before declining by late August. Throughout all years, larval activity generally ended by October; however, some larvae were still present as late as mid-November 2023.



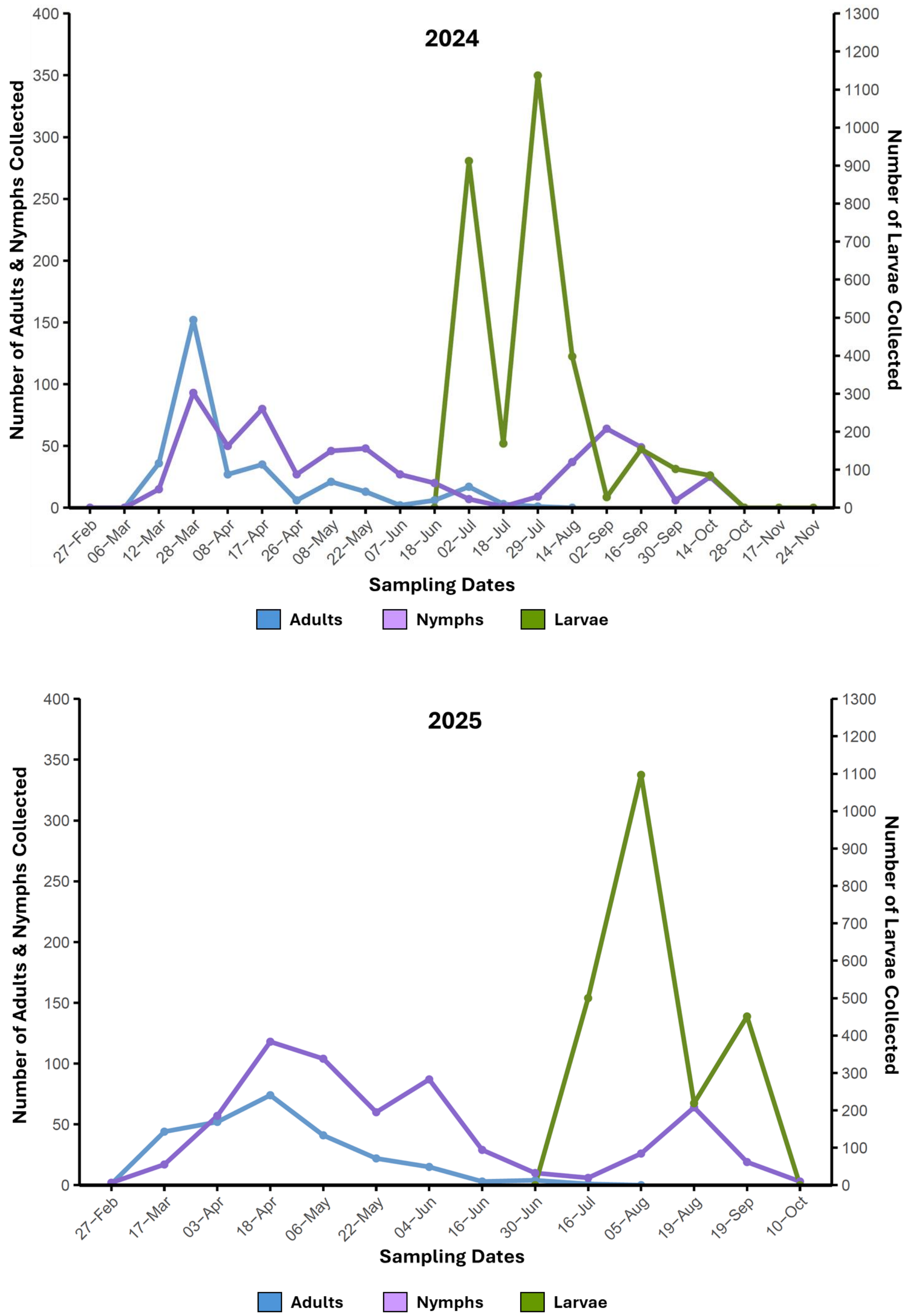


Figure 2.2. Multi-year patterns of *Amblyomma americanum* life stages in the Kansas Flint Hills (2023-2024).

The three panels show the total numbers of adults (blue line), nymphs (purple line), and larvae (green line) collected biweekly from February to November. Adults peaked in spring, nymphs dominated from late spring through summer, and larvae emerged in mid-to-late summer.

Influence of environmental factors on tick abundance

Seasonal patterns of *A. americanum* abundance were influenced by variations in temperature and relative humidity over the three years of sampling (Figures 2.3-2.5). Adults were the first tick stage to become active in early spring when minimum temperatures rose above freezing (5-10 °C) and relative humidity remained between 60 to 80% (Figure 2.3). Peaks in adult activity generally occurred during moderate spring conditions, when minimum temperatures were around 10-15 °C, maximum temperatures reached 20-27 °C, and humidity was relatively high (>65%). When the temperatures increased beyond 30 °C and the relative humidity was below 60% in early summer, adult numbers started to decline. These patterns suggest that heat and lower humidity reduce adult survival and host-seeking behavior.

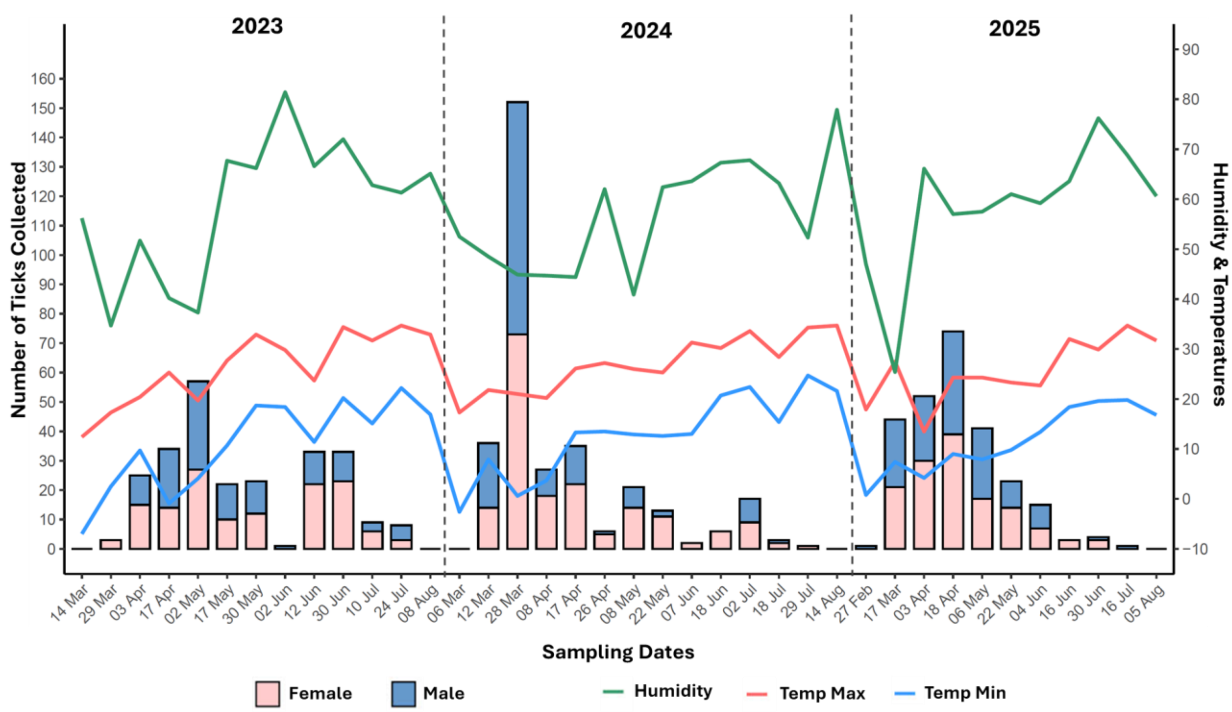


Figure 2.3. Adult *Amblyomma americanum* activity in relation to environmental conditions in the Kansas Flint Hills (2023-2025).

Stacked bars represent the number of adult females (pink) and males (blue) collected biweekly. Lines show relative humidity (green), maximum daily temperature (red), and minimum daily temperature (blue). Adult peaks occurred in spring under mild temperatures and higher humidity, followed by sharp declines as summer conditions became hotter and drier.

Nymphal activity was highly sensitive to changes in environmental conditions, showing clear variation among the sampling years (Figure 2.4). In 2023, the highest peak occurred in early May (over 350 ticks), when minimum temperatures ranged from 12 to 15 °C, maximum temperatures reached 25-28 °C, and relative humidity stayed near 70%. These conditions appeared to support high nymphal survival and questing activity. In contrast, the early spring of 2024 was characterized by cooler and drier conditions, with minimum temperatures ranging from 5 to 10 °C, maximum temperatures of 18-22 °C, and a relative humidity often below 60%. Under these less favorable conditions, nymphal abundance was lower, and activity was extended longer into late summer at reduced intensity. In 2025, nymphal numbers increased again in April (over 110 ticks), when the minimum temperature was 10 to 12 °C, the maximum temperature was 20-24 °C, and relative humidity remained around 65-70 %. Overall, these results indicate that moderate spring temperatures and consistent humidity promote strong nymphal activity and numbers, whereas cooler or drier conditions during spring tend to suppress abundance and prolong the activity period.

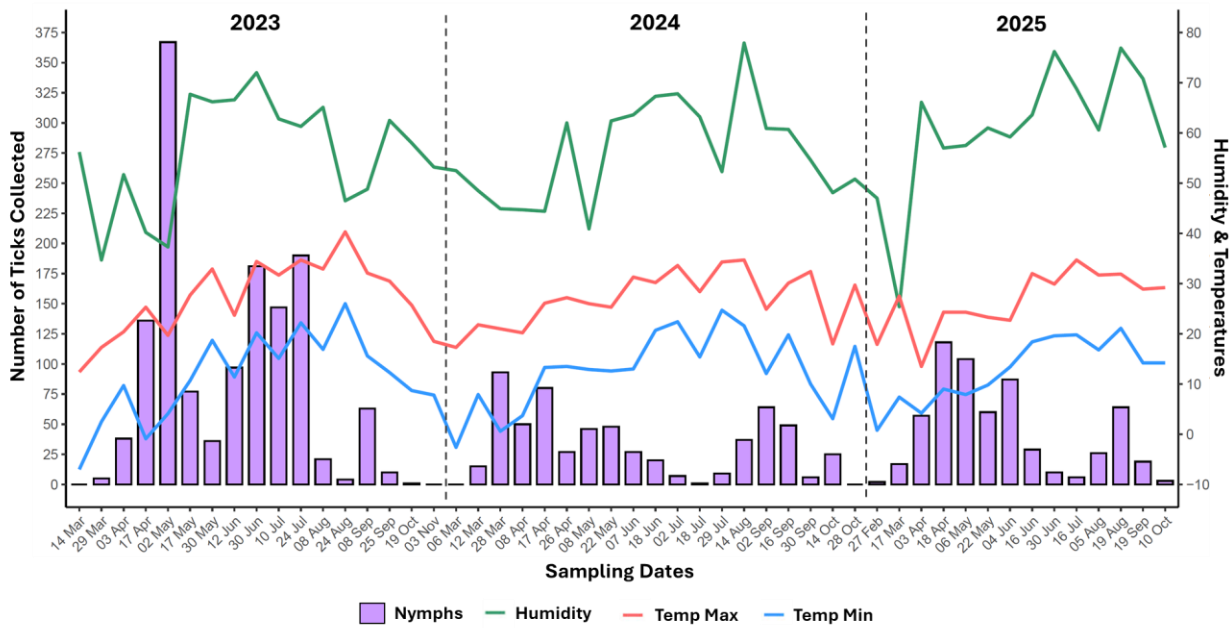


Figure 2.4. Seasonal activity of nymphal *Amblyomma americanum* in relation to temperature and humidity in the Kansas Flint Hills (2023-2025).

Bars represent the number of nymphs collected biweekly. Lines show relative humidity (green), maximum daily temperature (red), and minimum daily temperature (blue). Nymphal peaks occurred in spring under moderate temperatures and higher humidity, with interannual variation in magnitude and duration reflecting climatic differences among years.

Larval activity showed a strong association with the warm and humid conditions of late summer, with peaks corresponding to periods of high humidity and moderate to high temperatures (Figure 4). In 2023, the largest larval peak occurred in late August, when maximum temperatures were approximately 28-30 °C, minimum temperatures were around 18-20 °C, and relative humidity remained near 70%. These conditions provided an optimal microclimate for larval emergence and survival. In 2024, overall larval abundance was lower, with smaller peaks in July and August. During this period, relative humidity was lower (60-65%) and maximum temperatures were near 30 °C, conditions that may have increased desiccation stress and reduced survival. In 2025, larval abundance reached higher numbers in early August, under maximum temperatures of 27-29 °C, minimum temperatures around 18 °C, and relative humidity between 65 and 70%. These findings suggest that larval success is highly influenced by yearly climatic variability, particularly the balance between heat and moisture that determines the risk of desiccation and survival.

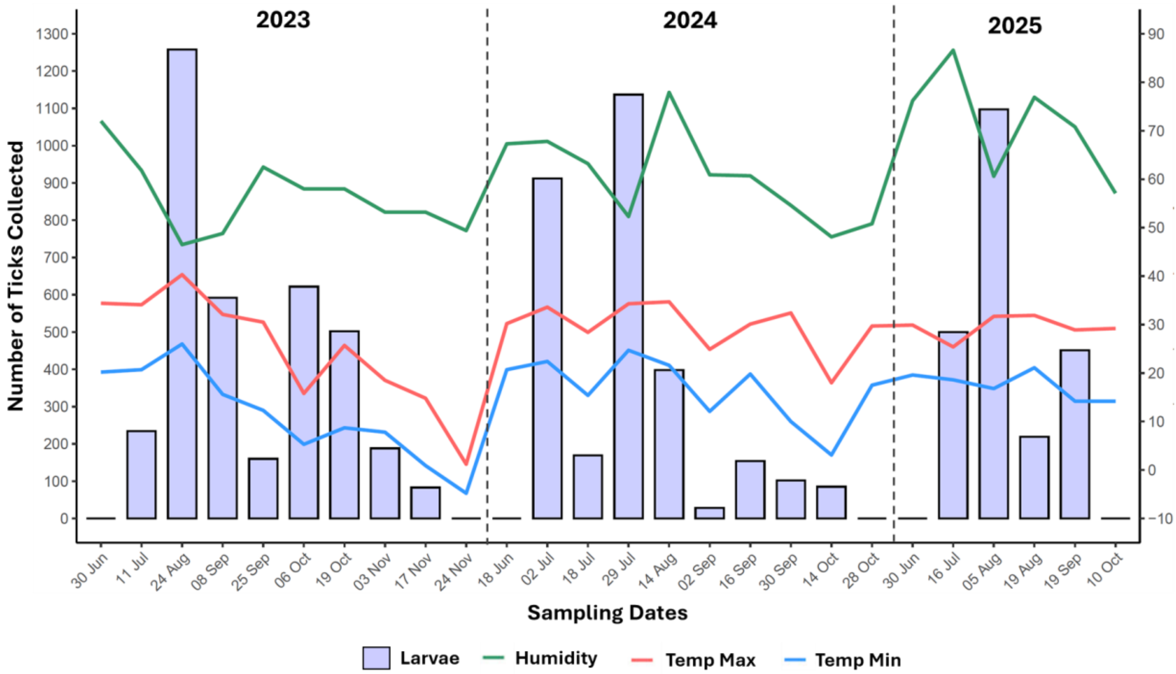


Figure 2.5. Seasonal activity of larval *Amblyomma americanum* in relation to the environmental conditions of the Kansas Flint Hills (2023-2025).

Bars represent the number of larvae collected biweekly. Lines show relative humidity (green), maximum daily temperature (red), and minimum daily temperature (blue). Larvae emerged in July and peaked in late summer, with the highest abundance in August 2023, under warm temperatures and high humidity. In contrast, 2024 and 2025 showed lower and shorter peaks, linked to drier conditions.

Schematic model of *Amblyomma americanum* life cycle

Field data collected over three consecutive years indicate that the life cycle of *A. americanum* typically takes about two or three years to complete (Figure 2.6). Adults lay eggs in late spring or early summer of the first year, and larvae hatch and are active from mid-July through October. After obtaining a blood meal, larvae molt into nymphs, which overwinter and emerge the following spring, with activity extending from early March through October of the second year.

Then, these nymphs develop into adults, which overwinter and reappear the following spring, completing the cycle during the third year (Figure 2.6).

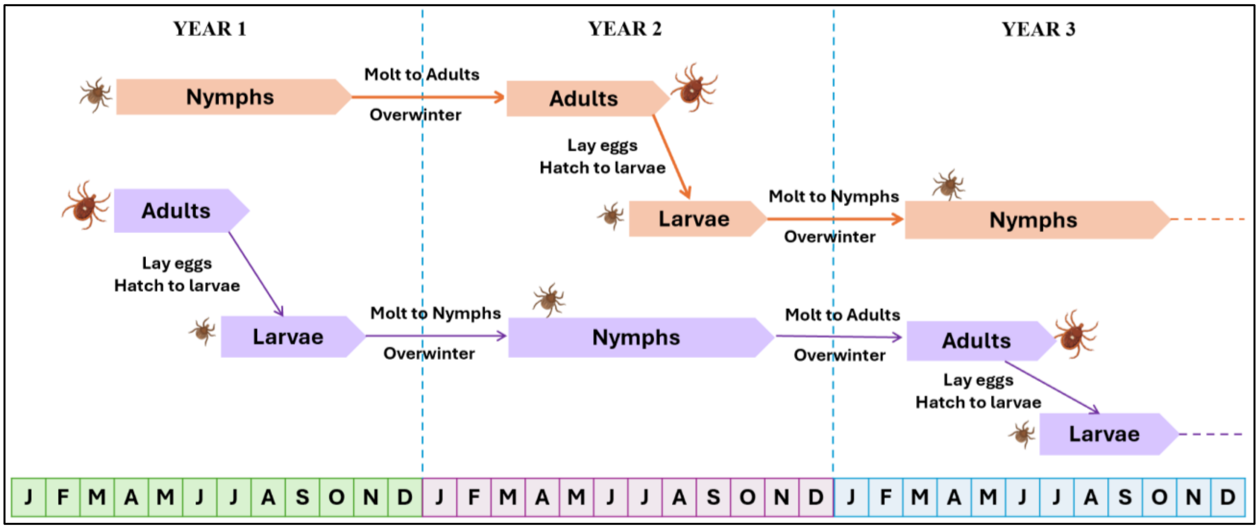


Figure 2.6. Schematic model of *Amblyomma americanum* life cycle based on three years of observations.

Discussion

Across the three years of sampling in the Kansas Flint Hills, the seasonal pattern of *A. americanum* followed a clear and consistent sequence. Adults were active first in early spring, followed by nymphs in late spring and summer, and larvae emerging in mid-to-late summer. Although this pattern remained stable from year to year, the tick numbers fluctuated considerably, especially among nymphs and larvae. Similar seasonal cycles have been reported in other regions of the United States; however, the exact timing and intensity of activity often vary depending on the geographic locations.

For example, adults typically appear in February in Mississippi, in March in Missouri, in Oklahoma, and northwestern Florida (Jackson et al. 1996, Cilek and Olson 2000, Kollars Jr et al. 2000, Bouzek et al. 2013). In our study, adult activity began earlier each year than previously reported, with first detections in mid-March 2023, early March 2024, and mid-February 2025. Similar early emergence patterns have been described in Oklahoma and the Central Midwest, where warmer winters and milder spring temperatures (2.77-20.55ºC) could enhance overwinter survival and trigger earlier questing behavior (Raghavan et al. 2021, Noden et al. 2022, McClung et al. 2023). These findings suggest that increasing temperatures may be shifting the phenology of *A. americanum* toward earlier seasonal activity in the Great Plains.

Adult abundance in this study peaked from April to June, which is consistent with reports from the southeastern United States, where adults are most active in spring and early summer (Cilek and Olson 2000, Kollars Jr et al. 2000, Gleim et al. 2014). However, in northern states such as Delaware, adult peak activity occurs in June, which is later compared to our observations (Kennedy and Marshall 2021). In the Kansas Flint Hills, adult activity declined by July, earlier than in most regional studies, where adults remained until August. This pattern may reflect an earlier onset of activity, suggesting that when adults emerge sooner, transitions between life stages also occur earlier within the same year.

Nymphal activity extended from April through October, lasting slightly longer than reported in Oklahoma and Missouri, where nymphs are typically active from April to September, and similar to patterns observed in Mississippi, where nymphal questing extends from May to October (Jackson et al. 1996, Kollars Jr et al. 2000, Bouzek et al. 2013). The seasonal pattern of nymphs may follow either a unimodal or bimodal distribution, with one peak in some years and two in others (Kollars Jr et al. 2000). Peaks commonly occur in May and July, which was reported in Oklahoma, Missouri, and in our dataset (Kollars Jr et al. 2000, Bouzek et al. 2013). In this study, larval activity began in July, consistent with observations from Mississippi and Missouri. In contrast, studies from Florida have documented larval emergence starting in June and continuing until November, with a peak in July (Cilek and Olson 2000). In the Kansas Flint Hills, the highest larval abundance occurred in August, a finding also reported in Tennessee and Missouri (Jackson et al. 1996, Kollars Jr et al. 2000, Bouzek et al. 2013). Across all studies, larval numbers consistently declined by October or November, indicating that late-summer emergence, followed by fall decline, is a stable characteristic of *A. americanum* phenology throughout its range.

The development of *A. americanum* is strongly influenced by environmental factors such as temperature and relative humidity, because most of its life cycle occurs off the host (Jackson et al. 1996, Springer et al. 2014, Ma et al. 2021, Bellman et al. 2024). In this study, adult activity began when minimum temperatures were around 10 °C and peaked at maximum temperatures of 20 to 27 °C under humidity of 60 to 80%. Adult abundance declined when temperatures exceeded 30 °C and relative humidity fell below 60%. Similar thresholds have been observed in Oklahoma, where the tick survival rate decreased significantly when temperatures exceeded 30 °C and humidity dropped below 65% (Koch 1984).

Nymphal activity followed a similar pattern. In 2023 and 2025, nymphs were most abundant during cool, moist spring periods, with minimum temperatures between 10 and 15 °C, maximum temperatures of 20-28 °C, and relative humidity above 65%. In contrast, in 2024, nymphal abundance was reduced, and activity extended later into the summer at a lower intensity, when the relative humidity dropped below 60%. Similar responses have been reported in other studies, where nymphal host-seeking and survival were reduced under drier conditions (Mangan et al. 2022).

Among all the tick stages, larvae are the most sensitive to moisture conditions (Jackson et al. 1996). In 2023, the largest larval cohort was observed during late summer when temperatures were warm and relative humidity was around 70%. In contrast, the larval peaks in 2024 and 2025 were smaller and shorter in duration, even though temperatures were similar. Reduced rainfall and fluctuating humidity likely increased desiccation stress and lowered survival. These results indicate that larval survival is strongly dependent on high humidity and moderate to warm temperatures, which help avoid desiccation (Mangan et al. 2022, Bellman et al. 2024).

Field data from this study support a two-year life cycle of *A. americanum* in the Kansas Flint Hills, consistent with the studies conducted in northeast Missouri (Bouzek et al. 2013). During the first year, adults lay eggs in late spring or early summer, and larvae hatch and begin questing activity from late July through October. Because unfed larvae have a low probability of surviving the winter, they must either feed and overwinter or molt into nymphs before entering dormancy (Sonenshine and Levy 1971, Koch 1984, Ma et al. 2021, Foré et al. 2023). The following spring, nymphs reappear as early as March and remain active until October, or in some regions until November. By late in the year, engorged nymphs molt into adults, which overwinter and continue the cycle the next year (Bouzek et al. 2013, Foré et al. 2023).

Evidence for behavioral diapause in adults comes from studies measuring hemoglobin (haem) concentration to estimate physiological age (Mangan et al. 2020). Adults collected in late spring and early summer showed low haem concentrations, suggesting they are older ticks that did not feed before overwintering and began host-seeking after emerging from dormancy. After feeding, females lay eggs during June or July, completing their cycle.

Because adults and larvae occur in different years than nymphs, *A. americanum* population within the same area may represent two overlapping cohorts. Although no genetic or genomic studies have yet confirmed this possibility. Further research comparing genetic variation among ticks collected in different seasons within the same geographic region is needed to determine whether temporal (allochronic) separation contributes to population structuring.

Understanding the life cycle of *A. americanum* and how it responds to environmental variation is important for both ecological and management purposes. From an ecological perspective, the timing of each life stage reflects interactions with hosts: adults and nymphs are most active in spring, increasing the risk of exposure to humans and livestock, while larvae dominate in late summer, feeding primarily on small mammals that maintain pathogen transmission cycles (Childs and Paddock 2003). From a management perspective, identifying seasonal activity peaks enables producers and public health officials to know the best time to apply tick control strategies, such as acaricide application, livestock treatments, and prescribed burning. In the tallgrass prairie ecosystem of the Kansas Flint Hills, where prescribed fire is a central management practice, synchronizing burn schedules with tick phenology could help to reduce tick populations and minimize host-tick contact (Cully 1999, Gleim et al. 2014). Finally, linking seasonal dynamics with environmental factors provides a foundation for predicting how *A. americanum* populations may respond to future climate changes and for developing adaptive strategies to mitigate the risks of tick-borne diseases.

Conclusion

This study underscores the importance of multi-year monitoring in tallgrass prairie ecosystems, where annual variations likely reflect how weather patterns and land management influence tick development and survival. Adults were most active under cooler and moist conditions in early spring. Nymphs reached their highest numbers during spring and summer, when temperatures were moderate and humidity remained high, and larvae were most abundant in late summer when heat is balanced by adequate humidity. Together, these findings demonstrate that interannual differences in relative humidity and temperature are key drivers of *A. americanum* population dynamics, which emphasizes the importance of long-term ecological studies for understanding and managing tick populations.

References

Barré N, Garris G, Lorvelec O. 1997. Field sampling of the tick *Amblyomma variegatum* (Acari: *Ixodidae*) on pastures in Guadeloupe; attraction of CO2 and/or tick pheromones and conditions of use. Experimental & applied acarology 21:95-108. https://doi.org/10.1023/b:appa.0000031788.88306.77.

Bellman S, Fausett E, Aeschleman L et al. 2024. Mapping the distribution of *Amblyomma americanum* in Georgia, USA. Parasites & Vectors 17(1):62. https://doi.org/10.1186/s13071-024-06142-7.

Bouzek D, Foré S, Bevell J et al. 2013. A conceptual model of the *Amblyomma americanum* life cycle in northeast Missouri. Journal of Vector Ecology 38(1):74-81. https://doi.org/10.1111/j.1948-7134.2013.12011.x.

Childs JE, Paddock CD. 2003. The ascendancy of *Amblyomma americanum* as a vector of pathogens affecting humans in the United States. Annual review of entomology 48(1):307-337. https://doi.org/10.1146/annurev.ento.48.091801.112728.

Cilek J, Olson M. 2000. Seasonal distribution and abundance of ticks (Acari: *Ixodidae*) in northwestern Florida. Journal of Medical Entomology 37(3):439-444. https://doi.org/10.1093/jmedent/37.3.439.

Cobos ME, Winters T, Martinez I et al. 2024. Modeling spatiotemporal dynamics of *Amblyomma americanum* questing activity in the central Great Plains. Plos one 19(10):e0304427. https://doi.org/10.1371/journal.pone.0304427.

Commins SP, James HR, Kelly LA et al. 2011. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-α-1, 3-galactose. Journal of Allergy and Clinical Immunology 127(5):1286-1293. e1286. https://doi.org/10.1016/j.jaci.2011.02.019.

Cully JF. 1999. Lone star tick abundance, fire, and bison grazing in tallgrass prairie. Rangeland Ecology & Management/Journal of Range Management Archives 52(2):139-144. https://doi.org/10.2307/4003507.

Dubie TR, Grantham R, Coburn L et al. 2017. Pictorial key for identification of immature stages of common ixodid ticks found in pastures in Oklahoma. Southwestern Entomologist 42(1):1-14. https://doi.org/10.3958/059.042.0101.

Durden LA, Beati L. 2013. Modern tick systematics. Biology of ticks 1:17-58. https://doi.org/10.1016/s0195-5616(91)50001-2.

Foré SA, Mangan MJ, Mantia AM et al. 2023. Multiple physiological cohorts comprise the seasonal activity of wild *Amblyomma americanum* (Acari: *Ixodidae*) nymphs. Ticks and Tick-borne Diseases 14(2):102091. https://doi.org/10.1016/j.ttbdis.2022.102091.

Gleim ER, Conner LM, Berghaus RD et al. 2014. The phenology of ticks and the effects of long-term prescribed burning on tick population dynamics in southwestern Georgia and northwestern Florida. PLoS One 9(11):e112174. https://doi.org/10.1371/journal.pone.0112174.

Haile D, Mount G. 1987. Computer simulation of population dynamics of the lone star tick, *Amblyomma americanum* (Acari: *Ixodidae*). Journal of Medical Entomology 24(3):356-369. https://doi.org/10.1093/jmedent/24.3.356.

Jackson LK, Gaydon DM, Goddard J. 1996. Seasonal activity and relative abundance of *Amblyomma americanum* in Mississippi. Journal of Medical Entomology 33(1):128-131. https://doi.org/10.1093/jmedent/33.1.128.

Keirans JE, Litwak TR. 1989. Pictorial key to the adults of hard ticks, family *Ixodidae* (*Ixodida: Ixodoidea*), east of the Mississippi River. Journal of Medical Entomology 26(5):435-448. https://doi.org/10.1093/jmedent/26.5.435.

Kennedy AC, Marshall E. 2021. Lone star ticks (*Amblyomma americanum*): an emerging threat in Delaware. Delaware journal of public health 7(1):66. https://doi.org/10.32481/djph.2021.01.013.

Knapp AK, Briggs JM, Hartnett DC et al. 1998. Grassland dynamics: long-term ecological research in tallgrass prairie. 1.

Koch H, McNew R. 1981. Comparative catches of field populations of lone star ticks (*Amblyomma americanum*) by CO2 (carbon dioxide) emitting dry-ice, dry-chemical, and animal-baited devices (Oklahoma). Annals of the Entomological Society of America (USA). https://doi.org/10.1603/ME10275.

Koch HG. 1984. Survival of the lone star tick, *Amblyomma americanum* (Acari: *Ixodidae*), in contrasting habitats and different years in southeastern Oklahoma, USA. Journal of Medical Entomology 21(1):69-79. https://doi.org/10.1093/jmedent/21.1.69.

Kollars Jr TM, Oliver Jr JH, Durden LA et al. 2000. Host associations and seasonal activity of *Amblyomma americanum* (Acari: *Ixodidae*) in Missouri. Journal of Parasitology 86(5):1156-1159. https://doi.org/10.1645/0022-3395(2000)086[1156:HAASAO]2.0.CO;2.

Ludwig A, Ginsberg HS, Hickling GJ et al. 2016. A dynamic population model to investigate effects of climate and climate-independent factors on the lifecycle of *Amblyomma americanum* (Acari: *Ixodidae*). Journal of Medical Entomology 53(1):99-115. https://doi.org/10.1093/jme/tjv150.

Ma D, Lun X, Li C et al. 2021. Predicting the potential global distribution of *Amblyomma americanum* (Acari: *Ixodidae*) under near current and future climatic conditions, using the maximum entropy model. Biology 10(10):1057. https://doi.org/10.3390/biology10101057.

Mangan MJ, Foré SA, Kim H-J. 2020. Using haem concentration as a metric of physiological age to infer demographic structure in natural field and forest populations of host-seeking *Amblyomma americanum* adults. International Journal of Acarology 46(4):258-262. https://doi.org/10.1080/01647954.2020.1758776.

Mangan MJ, Foré SA, Kim H-J. 2022. Seasonal changes in questing efficiency of wild *Amblyomma americanum* (Acari: *Ixodidae*) nymphs. Ticks and Tick-borne Diseases 13(5):101988. https://doi.org/10.1016/j.ttbdis.2022.101988.

McClung KL, Sundstrom KD, Lineberry MW et al. 2023. Seasonality of *Amblyomma americanum* (Acari: *Ixodidae*) activity and prevalence of infection with tick-borne disease agents in north central Oklahoma. Vector-Borne and Zoonotic Diseases 23(11):561-567. https://doi.org/10.1089/vbz.2023.0009.

Ng'eno E, Alkishe A, Romero-Alvarez D et al. 2024. Phenology of five tick species in the central Great Plains. Plos one 19(5):e0302689. https://doi.org/10.1371/journal.pone.0302689.

Noden BH, Dubie TR, Henriquez BE et al. 2022. Seasonality of ticks and prevalence of *Rickettsiae* species in *Dermacentor variabilis* and *Amblyomma maculatum* across Oklahoma pastures. Journal of Medical Entomology 59(3):1033-1041. https://doi.org/10.1093/jme/tjac028.

Petry WK, Foré SA, Fielden LJ et al. 2010. A quantitative comparison of two sample methods for collecting *Amblyomma americanum* and *Dermacentor variabilis* (Acari: *Ixodidae*) in Missouri. Experimental and Applied Acarology 52:427-438. https://doi.org/10.1007/s10493-010-9373-9.

Raghavan RK, Peterson AT, Cobos ME et al. 2019. Current and future distribution of the lone star tick, *Amblyomma americanum* (L.)(Acari: *Ixodidae*) in North America. PloS one 14(1):e0209082. https://doi.org/10.1371/journal.pone.0209082.

Raghavan RK, Goodin DG, Hanzlicek GA et al. 2016. Maximum entropy-based ecological niche model and bio-climatic determinants of lone star tick (*Amblyomma americanum*) niche. Vector-Borne and Zoonotic Diseases 16(3):205-211. https://doi.org/10.1089/vbz.2015.1837.

Raghavan RK, Koestel ZL, Boorgula G et al. 2021. Unexpected winter questing activity of ticks in the Central Midwestern United States. PLoS One 16(11):e0259769. https://doi.org/10.1371/journal.pone.0259769.

Sagurova I, Ludwig A, Ogden NH et al. 2019. Predicted northward expansion of the geographic range of the tick vector *Amblyomma americanum* in North America under future climate conditions. Environmental Health Perspectives 127(10):107014. https://doi.org/10.1289/EHP5668.

Savage HM, Godsey Jr MS, Panella NA et al. 2016. Surveillance for Heartland virus (*Bunyaviridae: Phlebovirus*) in Missouri during 2013: first detection of virus in adults of *Amblyomma americanum* (Acari: *Ixodidae*). Journal of Medical Entomology 53(3):607-612. https://doi.org/10.1093/jme/tjw028.

Sonenshine DE, Levy GF. 1971. The ecology of the lone star tick, *Amblyomma americanum* (L.), in two contrasting habitats in Virginia (Acarina: *Ixodidae*). Journal of Medical Entomology 8(6):623-635. https://doi.org/10.1093/jmedent/8.6.623.

Springer YP, Eisen L, Beati L et al. 2014. Spatial distribution of counties in the continental United States with records of occurrence of *Amblyomma americanum* (*Ixodida: Ixodidae*). Journal of Medical Entomology 51(2):342-351. https://doi.org/10.1603/me13115.

Stromdahl E, Hickling G. 2012. Beyond Lyme: Aetiology of tick-borne human diseases with emphasis on the South-Eastern United States. Zoonoses and public health 59:48-64. https://doi.org/10.1111/j.1863-2378.2012.01475.x.

Zimmerman RH, McWherter GR, Bloemer SR. 1987. Role of small mammals in population dynamics and dissemination of *Amblyomma americanum* and *Dermacentor variabilis* (Acari: *Ixodidae*) at Land Between the Lakes, Tennessee. Journal of Medical Entomology 24(3):370-375. https://doi.org/10.1093/jmedent/24.3.370.

Chapter 3 - Reducing tick populations through prescribed burning

Abstract

As obligatory blood-feeding ectoparasites, ticks are capable of transmitting pathogens to both humans and animals. Current tick control methods, which rely on the application of acaricidal chemicals, are not always effective, due in part to ticks spending most of their life cycle off the animal host. The intentional burning of grasslands (prescribed burning) has been proposed as a potential method for decreasing tick abundance during their off-host periods; however, its efficacy as a tick control mechanism requires further evaluation. This study aimed to explore the impact of prescribed burning on tick populations in native warm-season grasslands of the Kansas Flint Hills. Pastures were burned annually in the spring, summer, or fall four times over a five-year period. After these treatments, ticks were collected biweekly from March to November 2023 and 2024 and identified morphologically utilizing a stereoscope and published keys. Results demonstrated significantly lower tick populations in fire-treated pastures compared to non-burned pasture sites ($P<0.0001$). In addition, tick populations were significantly lower in areas burned in the spring compared to those burned in the summer ($P = 0.0105$) and fall ($P < 0.0001$). These findings suggest that regular prescribed burning maintains lower tick populations in pastures that are burned annually. Furthermore, managing tick numbers may help lower the economic impact associated with tick-borne diseases.

Introduction

Ticks are the most important obligatory blood-feeding ectoparasites of livestock and humans (Anderson and Magnarelli 2008). These ectoparasites are widespread and can be found in diverse habitats, ranging from forests and grasslands to urban areas. Ticks are known for their ability to transmit a wide variety of pathogenic agents, including bacteria, viruses, and protozoa

(Brites-Neto et al. 2015). Ticks are the second vector group responsible for transmitting a significant number of pathogens to humans, play a primary role in the transmission of diseases to animals (Brites-Neto et al. 2015).

Ticks and their associated pathogens can cause significant mortality and morbidity in animals, resulting in billions of dollars in losses to the global livestock industry. In the United States, ticks affecting livestock inflict substantial economic losses (McCluskey 2018). According to the USDA, an extended cattle tick outbreak could result in financial consequences surpassing $1.2 billion, accounting for control costs alone. They cause direct damage (blood loss, damage to the skin, etc.) as well as indirect harm through secondary infections in the bitten area and the transmission of pathogens (Abubakar and Perera 2019).

Previous studies in Kansas have identified several common tick species, including *Amblyomma americanum* (lone star tick), *Amblyomma maculatum* (Gulf coast tick), *Dermacentor variabilis* (American dog tick), *Dermacentor albipictus* (winter tick), *Ixodes scapularis* (blacklegged tick or deer tick), and *Rhipicephalus sanguineus* (brown dog tick) (Hroobi et al. 2021, Ng'eno et al. 2024, Olds 2025). These tick species represent a growing challenge for both human and animal health because they can cause diseases such as ehrlichiosis, anaplasmosis, Lyme disease, Rocky Mountain spotted fever, and tularemia (Hroobi et al. 2021, Ng'eno et al. 2024). In addition, the continued expansion of tick ranges has allowed new species to establish in previously unaffected areas. For example, *Haemaphysalis longicornis* (Asian longhorned tick), an invasive species capable of causing bovine theileriosis, a disease that can severely impact cattle production (Molaei et al. 2022). The increasing number of tick-borne disease cases each year is closely linked to climate change, habitat modification, and host movement and availability. Because these factors are difficult to control, developing effective tick management strategies is necessary to reduce tick populations and mitigate the risk of pathogen transmission to humans and animals.

Tick control in livestock has relied mainly on the use of on-animal acaricides; however, chemical control has significant deleterious effects, including the generation of pesticide resistance, high levels of mammalian and environmental toxicity, and the killing of non-target organisms (De la Fuente et al. 2007, Polito et al. 2013). Additionally, on-animal acaricides can only target the tick while it is on the host, but do not extend to time spent on alternative hosts (such as small wild mammals) or in the environment (molting, laying eggs, or overwintering). As a result, a large proportion of the tick life cycle escapes control, emphasizing the need for alternative non-chemical methods for tick control that can be effective and sustainable.

Prescribed burning is a managed fire practice applied under controlled conditions to achieve specific ecological or land management goals (Grebner et al. 2013). This type of disturbance provides multiple benefits to the environment, including improving vegetation structure, limiting woody plant encroachment, and maintaining open grassland habitats. Fire can also help manage plant diseases and arthropod pests by removing or reducing the vegetation that supports them. Fire disrupts the life cycle of some pests and diseases, contributing to overall ecosystem health (Gallagher et al. 2022).

Prescribed fire has been shown to influence tick populations by altering key microhabitat conditions necessary for their survival. Reductions in leaf litter following burns are often associated with lower tick abundance, likely due to changes in soil temperature and exposure, reducing the overwintering success (Allan 2009, Gallagher et al. 2022). Fire also reduces humidity and increases surface temperatures, creating drier conditions that can lead to desiccation stress during dry periods (Gleim et al. 2014, Gallagher et al. 2022). Through these combined effects, prescribed burning disrupts critical environmental conditions that support tick persistence, making it a promising component of integrated tick management strategies.

Controlled burning has been proposed as an alternative method for managing ticks in grazing systems, but results from previous studies have been inconsistent (Davidson et al. 1994b, Polito et al. 2013). Some studies found minimal reductions in tick abundance after burning, while others reported significant declines in tick populations immediately after treatment and up to one year post-application (Padgett et al. 2009, Willis et al. 2012, Gleim et al. 2014). Variability in these findings may be related to how fires are conducted, including differences in frequency, scale, and whether they involve single or repeated applications. When prescribed fire is applied once or inconsistently, then it may not capture the long-term effects that sustained burns can produce (Gleim et al. 2014). These studies may not accurately reflect the outcomes of prescribed burning across all ecosystems. For this reason, the present study explored the impact of sustained prescribed burning on tick populations in native warm-season grasslands of the Kansas Flint Hills.

Materials and methods

Sampling site

This project was conducted in the Kansas State University Beef Stocker Unit located west of Manhattan, Kansas (39°13’48.80N, 96°38’35.56W). The unit consists of around 450 hectares (ha) of native tallgrass ecosystem, divided into 18 grazing pastures with sizes between 12 to 30 ha. Pastures were grouped (six pastures per treatment) and assigned to three prescribed fire timing treatments: spring (April, ordinary day number 101 ± 6), summer (August, ordinary day number 237 ± 7), and fall (September/October, ordinary day number 275 ± 8) (Figure 3.1). Prescribed fires were carried out for four years before this study with authorization from Riley County Emergency Management in Manhattan, KS (permit no. 1488). In 2022, no burning was carried out due to a lack of adequate thatch. Within each prescribed fire treatment, we randomly selected three pastures for tick sampling, along with an unburned pasture that served as the control.

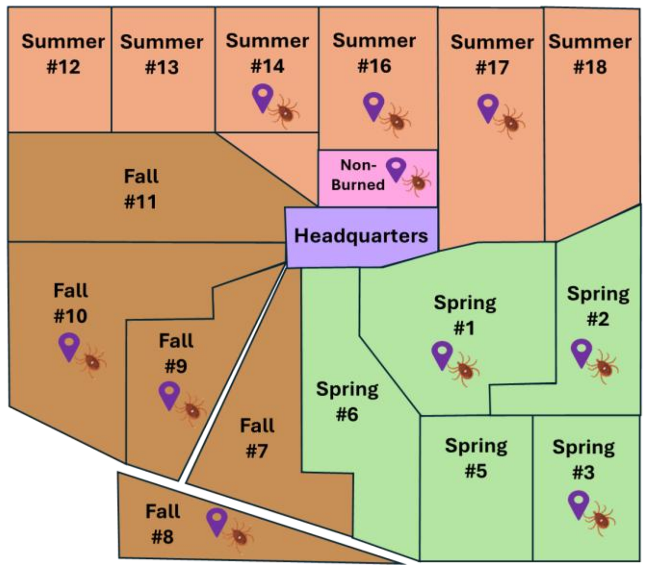


Figure 3.1. Map of the Kansas State University Stocker Unit, illustrating the 18 pastures divided into three groups based on prescribed fire timing (spring, summer, and fall). The location icon indicates the selected pastures for tick sampling as well as the non-burned field designated as control.

Tick collection and identification

In 2023 and 2024, ticks were collected biweekly from March to November. Permanent sampling sites were established to minimize microhabitat variation and potential sampling bias. Ticks from all life stages were trapped using drag sampling and dry ice baiting methods described in Chapter 2. Collected ticks were counted and identified to species and life stages based on morphological characteristics using a stereoscope and published identification keys.

Data analysis

We evaluated the effect of multi-year prescribed burning on tick populations by comparing the number of ticks collected in 2023 from three pastures treated with prescribed fire in spring, summer, and fall to those collected from an unburned pasture. We also assessed the local impact of prescribed fire within each pasture by comparing tick numbers from burned and unburned sections separated by 100 meters. To evaluate tick population recovery after a prescribed fire, we compared tick numbers from a pasture burned annually to those from a pasture burned approximately two years prior. For the analysis, each larval cluster was counted as a single tick, regardless of the number of larvae collected within it, to minimize data skew.

Statistical analyses were performed using Kruskal-Wallis non-parametric tests, Dunn's multiple comparisons, and Mann-Whitney tests, with a significance level set at 0.05 ($P<0.05$). All statistical analyses and plots were generated using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

Results

Local impact of prescribed burning

To investigate the local impact of a post-fire event, we analyzed the tick counts from burned and unburned patch areas, separated by 10 meters, within the same pasture during 2024. The variation in tick numbers was associated with sampling dates and sites because ticks were collected from three pastures with burned and unburned areas in each. The Mann-Whitney test showed significantly lower tick numbers in the burned areas relative to unburned areas ($U = 72.50$, $p = 0.0036$) (Figure 3.2). These results indicate that the effects of prescribed fire were confined to the burned areas, with significant changes in tick abundance observed only within these areas.

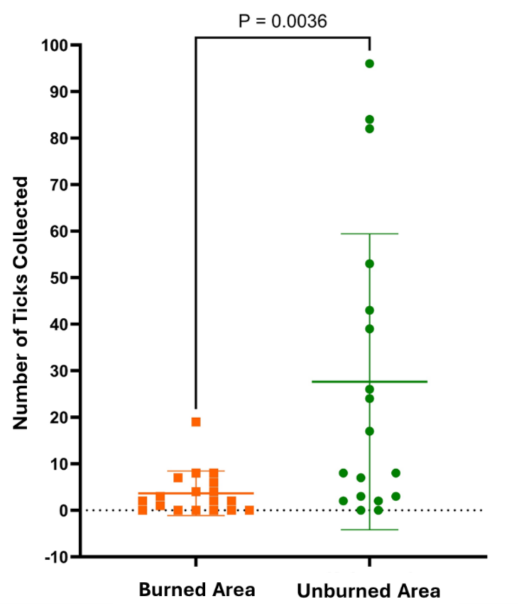


Figure 3.2. Comparison of tick abundance in burned and unburned areas within the same pasture to observe the local impact of prescribed burning.

Each dot represents the total number of ticks collected on each sampling day and site. Error bars indicate the standard deviation (SD), ns = not significant.

Impact of sustained prescribed burning

Over the sampling period, a total of 908 adult ticks, 2351 nymph ticks, and 3655 larval ticks were collected across all study sites (Table 3.1). The lone star tick (*Amblyomma americanum*) was the most frequently observed species, accounting for 99.3% of all collected ticks. In comparison, the American dog tick (*Dermacentor variabilis*) (0.65%) and the Gulf Coast tick (*Amblyomma maculatum*) (0.04%) were found less frequently.

Table 3.1. The number of ticks collected across three spring-, summer-, and fall-burned or unburned pastures.

Prescribed Fire Timing	Number of larvae	Number of nymphs	Number of adults			Total
	A. americanum	***A. americanum***	***A. americanum***	***D. variabilis***	***A. maculatum***	
Spring	3	52	48	1	0	**104**

Summer	7	565	216	1	0	**789**
Fall	6	129	100	7	2	**244**
Unburned	3639	1605	496	36	1	**5777**

No significant differences in tick numbers were observed within the fire treatment groups for spring ($H = 2.983$, $df = 2$, $p = 0.225$), summer ($H = 5.543$, $df = 2$, $p = 0.0626$), or the unburned control pasture ($H = 4.178$, $df = 2$, $p = 0.1238$). However, tick counts varied significantly between fall-burned pastures, with one pasture exhibiting a significantly higher tick count ($H = 7.358$, $df = 2$, $p = 0.0252$) (Table 3.2).

Table 3.2 Comparison of tick counts among pastures within each fire treatment or unburned control area.

Prescribed Fire Timing	H (Kruskal-Wallis test)	Degrees of freedom (df)	p-value
Spring	2.983	2	0.225
Summer	5.543	2	0.0626
Fall	7.358	2	0.0252
Unburned	4.178	2	0.1238

Significantly fewer ticks were collected from fire-treated sites than from non-burned control sites over the 10 months of the sampling period ($H= 55.57$; $df= 3$; $p<0.0001$) (Figure 3.3). While prescribed burning was equally effective in reducing tick populations in both tree line and grass areas (post-hoc Dunn's test, $p>0.9999$), pairwise comparisons using Dunn's test revealed significantly higher tick counts in unburned tree line sampling areas compared to grassland sampling areas ($p=0.0006$) (Figure 3.4 A, B, C).

When comparing seasons of burn (spring, summer, or fall), tick counts were highest in sites burned during summer compared to those burned in fall and spring (Table 3.1). Kruskal-Wallis analysis indicated that the timing of prescribed burning significantly influenced tick abundance

(H=19.74, df=2, p<0.0001), demonstrating a strong interaction between burning and season. Post-hoc pairwise comparison further showed that tick populations were significantly lower in areas burned in the spring compared to those burned in the summer (P = 0.0105) and fall (p < 0.0001) (Figure 3.5).

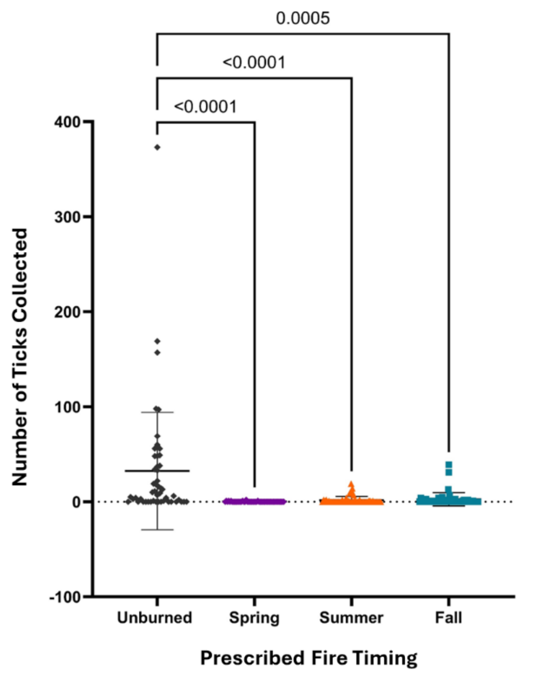


Figure 3.3. Prescribed burning significantly reduces tick populations.

Each dot represents the total number of ticks collected on each sampling day and site. Error bars indicate the standard deviation (SD), ns= not significant.

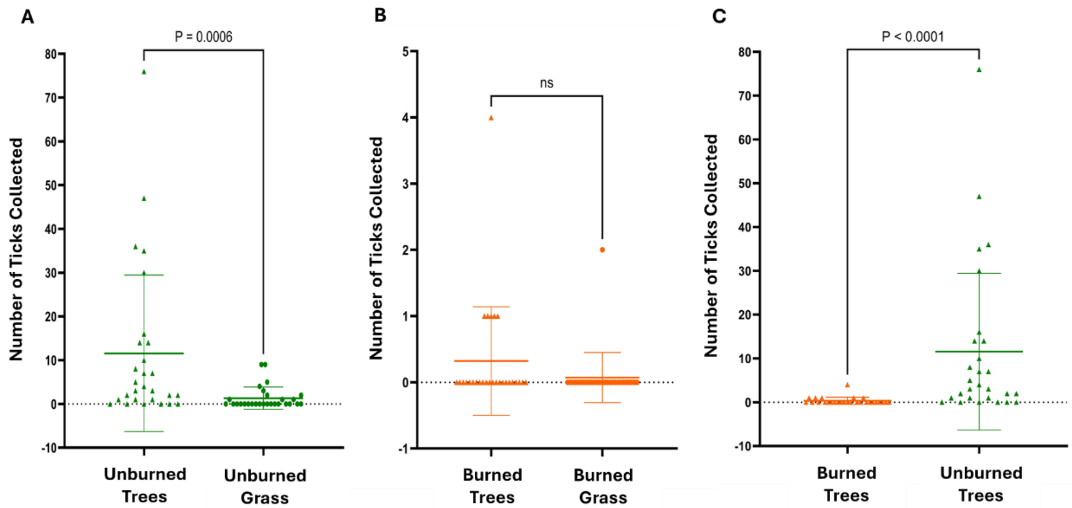


Figure 3.4. Variation in tick abundance across habitat and the influence of prescribed burning: tree line vs. grassland areas.

(A) Higher tick numbers in unburned tree line areas compared to unburned grassland. (B) Low tick numbers and no significant difference between burned tree line and grassland areas. (C) Prescribed burning effectively reduces tick populations in tree line areas. Each dot represents the total number of ticks collected on each sampling day and site. Error bars indicate the standard deviation (SD), ns = not significant.

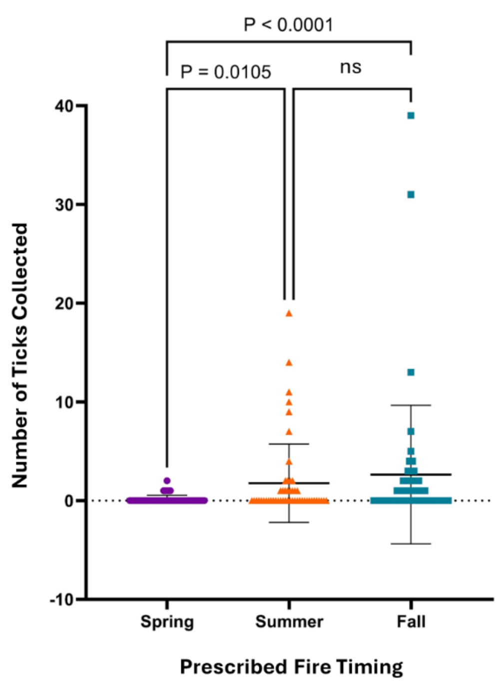


Figure 3.5. Tick numbers were significantly lower in spring-burned pastures compared to those burned in summer or fall.

Each dot represents the total number of ticks collected on each sampling day and site. Error bars indicate the standard deviation (SD), ns = not significant.

Tick population recovery without prescribed burning

Regular prescribed burning resulted in a reduced tick population in pastures burned annually. In contrast, pastures that had not undergone prescribed fire for approximately 600 days exhibited higher tick numbers, highlighting the role of fire in reducing tick populations (Figure 3.6). When burning ceased, a significantly higher tick population was collected in 2024 compared to 2023 ($p = 0.0147$). However, no significant difference was observed between tick populations from 2023 and 2024, where burning was maintained (>0.9999) (Figure 3.7A). Moreover, tick abundance did not differ significantly between pastures where prescribed burning had been discontinued and those that remained unburned for over five years ($p>0.9999$) (Figure 3.7B). These findings suggest that in the absence of prescribed fire, tick populations would rebound rapidly.

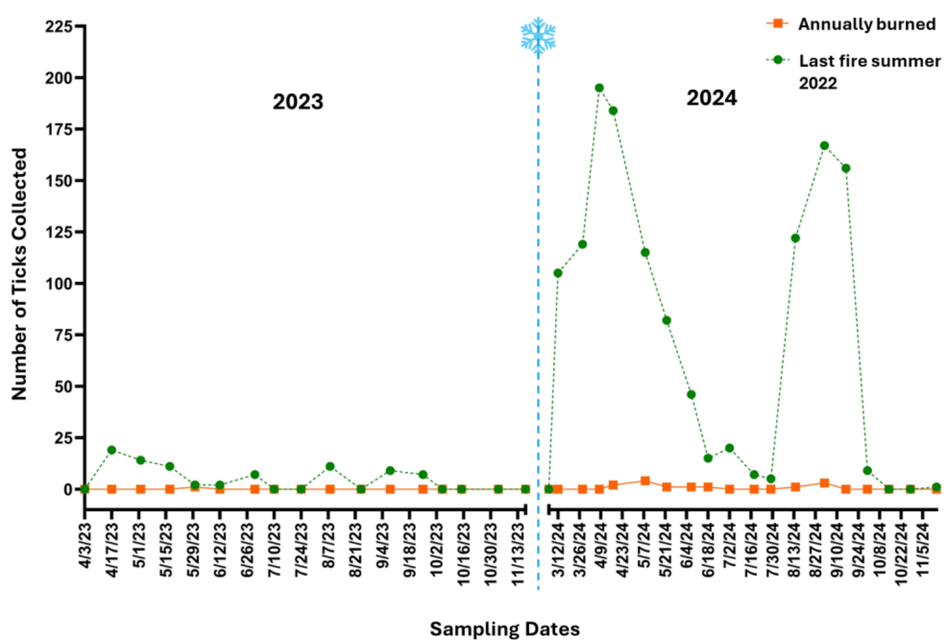


Figure 3.6 Tick population dynamics in annually burned vs. long-unburned pastures.

Showing lower tick numbers with frequent prescribed burning and a rebound where the prescribed fire was absent for ~600 days. Each dot represents the total number of ticks collected on each sampling day.

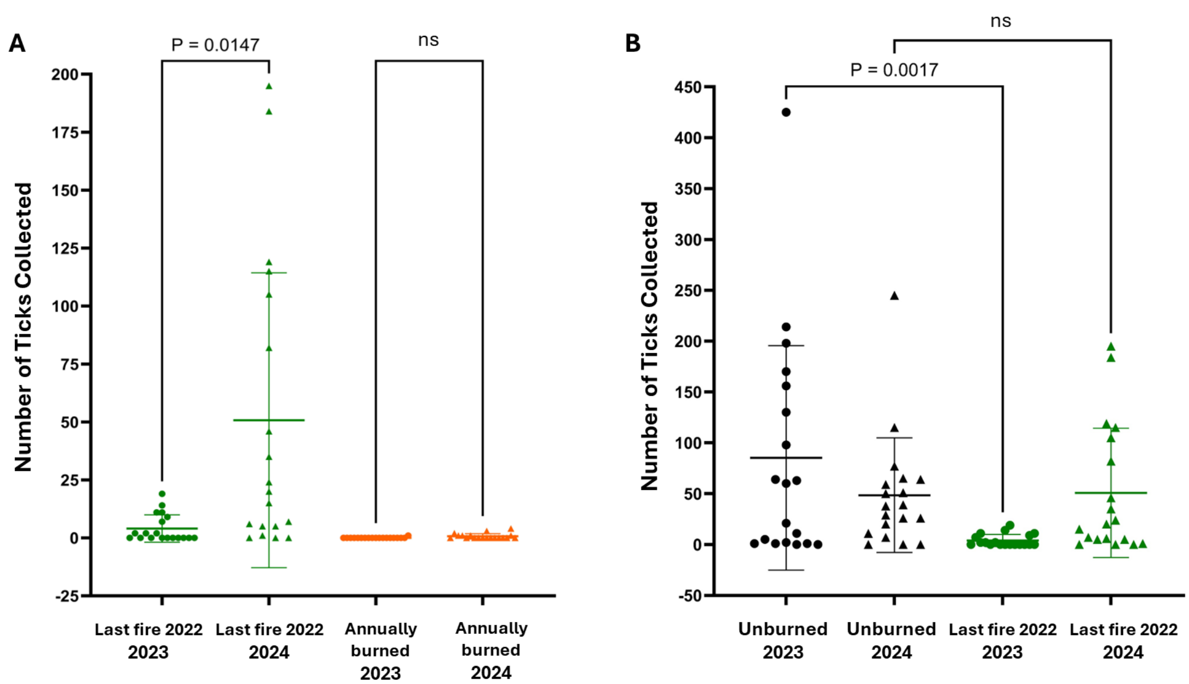


Figure 3.7 Recovery of tick populations in the absence of prescribed burning.

(A) Annual prescribed burning maintains low tick numbers, while discontinued burning leads to a population increase after 600 days. (B) Increase in tick population in pasture burned ~600 days ago, comparable to the control pasture unburned for over five years. Each dot represents the total number of ticks collected on each sampling day and site. Error bars indicate the standard deviation (SD), ns= not significant.

Discussion

Three different tick species were identified during this study: the lone star tick (*Amblyomma americanum*), the American dog tick (*Dermacentor variabilis*), and the Gulf Coast tick (*Amblyomma maculatum*). The lone star tick is broadly distributed across the eastern United States, ranging from central Texas, Oklahoma, and Kansas to the Atlantic coast as far north as Maine (CDC 2019, Raghavan et al. 2019). The American dog tick has a broad range across the central and eastern United States and has also been recorded in parts of California (CDC 2019, Boorgula et al. 2020). The Gulf Coast tick is often found in the southern states, including southeastern Kansas and Oklahoma (CDC 2019). These tick species are important vectors of several pathogens of medical and veterinary importance, such as *Rickettsia* spp., *Ehrlichia* spp., *Francisella tularensis*, *Anaplasma marginale*, Bourbon virus, and Heartland virus (Hecht et al. 2019, Higuita et al. 2021, Guizzo et al. 2022). Effective control of these vectors is critical in regions where these species are prevalent, particularly in the Kansas Flint Hills. This region is one of the last remaining tracts of tallgrass prairies in the United States and an important area for cattle production due to its high-quality forage (Anderson 1953, Towne and Craine 2016). Reducing tick populations in this ecosystem is not only essential for protecting human and animal health but also for enhancing cattle productivity and minimizing economic losses.

Among the three tick species found in the Kansas Flint Hills, *A. americanum* was the most abundant, representing 99.3% of the total ticks identified. Its high abundance was also observed in previous studies in another region of the United States, which emphasizes its aggressive questing and host-seeking behavior, and ability to move, locate, and attach to hosts (Gilliam et al. 2018, Marshall et al. 2025). This dominance suggests that *A. americanum* may compete with other tick species, such as *D. variabilis*, through advantages in habitat utilization, host acquisition, and environmental adaptability, potentially influencing tick community composition in the region (Stromdahl and Hickling 2012, Gleim et al. 2014).

Tick numbers were significantly higher in tree line areas compared to grassland areas. Similar findings were reported in southwestern Georgia and northwestern Florida, where *A. americanum* was most abundant in unburned, hardwood-dominated areas (Gleim et al. 2014). Semtner et al. (1971) also reported that adults and nymphs of this tick species were more commonly found in woodland habitats than in open prairie habitats. In our study, we collected more tick larvae in tree line sites than in grassland areas, likely due to lower oviposition and hatching success in tall-grass prairies versus oak-hickory forests (Allan 2009, Polito et al. 2013).

We collected significantly fewer ticks in areas treated with prescribed fire in mid-April (spring burns). This result supports earlier studies showing that spring fires remove large amounts of leaf litter and plant materials, creating environmental conditions that are less suitable for tick survival, which produces a reduction in the tick populations (Hoch et al. 1972, Davidson et al. 1994a, Hutchinson et al. 2005, Allan 2009). Additionally, one study conducted in the Missouri Ozarks found that spring burns reduced ground layer litter and vegetation by up to 73% (Hartman and Heumann 2003). Based on these findings, spring may be an ideal time to burn pastures and significantly impact tick abundance.

The spring application of prescribed fire may be particularly effective for managing *A. americanum*, whose life cycle typically lasts around two years. Overwintering adults and nymphs often emerge in early March, with adults initiating oviposition by late April. Nymphs remain active from April through September, while larvae are most active between July and October (Bouzek et al. 2013). Burning in early spring, before overwintering stages become fully active, targets ticks while they are still sheltering in leaf litter and less mobile, potentially increasing fire efficacy. In contrast, fires applied in summer or fall may be less effective because many nymphs and larvae are actively feeding on their host and can avoid direct exposure to heat. Therefore, synchronizing prescribed burn schedules with the seasonal activity of the vulnerable stages may maximize tick control success.

The impact of the fire was largely confined to areas that were directly burned. Significantly lower tick numbers were collected from burned areas compared to nearby unburned areas. Since *A. americanum* has limited mobility in all life stages and relies on questing and hunting to attach to a host (Hoch et al. 1972, Davidson et al. 1994a, Allan 2009). This mobility behavior makes them more vulnerable to environmental changes caused by prescribed burning. Interestingly, Gleim et al. (2014) observed that tick recolonization tends to occur more rapidly in smaller burns than in larger burn areas, suggesting that increasing the area treated could extend the period without ticks.

According to Cully (1999), prescribed burns create environmental conditions that suppress tick survival for about one to two years, reflecting their short-term to mid-term effectiveness. Our results follow a similar trend; tick numbers remained low for nearly 600 days after burning, then gradually returned to levels comparable to those in pastures that had not been burned for more than five years. Similar patterns of rapid rebound have been reported in other studies, especially when burn intervals are extended, and in some cases, tick populations even exceeded pre-burn levels (Allan 2009, Polito et al. 2013). These results suggest that while prescribed fire can be an effective tool for reducing tick numbers, its benefits are temporary without regular application. Therefore, incorporating frequent and well-timed fires is essential for maintaining long-term reductions in tick populations and mitigating the associated risk of tick-borne diseases.

Conclusion

Prescribed burning is an effective land management practice that significantly reduces tick populations and disrupts their life cycle by modifying the environmental conditions necessary for survival. Our findings demonstrate that annually prescribed burns maintained low tick abundance, while areas burned less frequently allowed recolonization over time. The effectiveness of fire in controlling tick populations is associated with the reduction of leaf litter and vegetation, which are critical microhabitats for ticks. Additionally, strategically timed burns, especially in the spring, can maximize the impact on tick mortality by targeting overwintering stages when ticks are most vulnerable.

In addition to decreasing tick populations, prescribed burning is a valuable tool in controlling the spread of tick-borne diseases that affect humans and animals. Ticks transmit pathogens responsible for diseases, such as Lyme disease, ehrlichiosis, and anaplasmosis, which can impact livestock, wildlife, and public health. To enhance the long-term success of prescribed burns, it is essential to apply fire treatments across larger areas, including tree line habitats, as these areas often serve as refuges for ticks and their hosts.

Reference

Abubakar M, Perera PK. 2019. *Ticks and Tick-Borne Pathogens, BoD–Books on Demand.*

Allan BF. 2009. Influence of prescribed burns on the abundance of *Amblyomma americanum* (Acari: *Ixodidae*) in the Missouri Ozarks. Journal of Medical Entomology 46(5):1030-1036. https://doi.org/10.1603/033.046.0509.

Anderson JF, Magnarelli LA. 2008. Biology of ticks. Infectious disease clinics of North America 22(2):195-215. https://doi.org/10.1016/j.idc.2007.12.006.

Barré N, Garris G, Lorvelec O. 1997. Field sampling of the tick *Amblyomma variegatum* (Acari: *Ixodidae*) on pastures in Guadeloupe; attraction of CO2 and/or tick pheromones and conditions of use. Experimental & applied acarology 21:95-108. https://doi.org/10.1023/b:appa.0000031788.88306.77.

Boorgula GD, Peterson AT, Foley DH et al. 2020. Assessing the current and future potential geographic distribution of the American dog tick, *Dermacentor variabilis* (Say) (Acari: *Ixodidae*) in North America. PLoS One 15(8):e0237191. https://doi.org/10.1371/journal.pone.0237191.

Bouzek D, Foré S, Bevell J et al. 2013. A conceptual model of the *Amblyomma americanum* life cycle in northeast Missouri. Journal of Vector Ecology 38(1):74-81. https://doi.org/10.1111/j.1948-7134.2013.12011.x.

Brites-Neto J, Duarte KMR, Martins TF. 2015. Tick-borne infections in human and animal population worldwide. Veterinary world 8(3):301. https://doi.org/10.14202/vetworld.2015.301-315.

CDC. 2019. Geographic distribution of ticks that bite humans. https://doi.org/www.cdc.gov/ticks/about/where-ticks-live.html.

Cully JF. 1999. Lone star tick abundance, fire, and bison grazing in tallgrass prairie. Rangeland Ecology & Management/Journal of Range Management Archives 52(2):139-144. https://doi.org/10.2307/4003507.

Davidson WR, Siefken DA, Creekmore LH. 1994a. Seasonal and annual abundance of *Amblyomma americanum* (Acari: *Ixodidae*) in central Georgia. Journal of Medical Entomology 31(1):67-71. https://doi.org/10.1093/jmedent/31.1.72.

Davidson WR, Siefken DA, Creekmore LH. 1994b. Influence of annual and biennial prescribed burning during March on the abundance of *Amblyomma americanum* (Acari: *Ixodidae*) in central Georgia. Journal of Medical Entomology 31(1):72-81. https://doi.org/10.1093/jmedent/31.1.72.

De la Fuente J, Almazán C, Canales M et al. 2007. A ten-year review of commercial vaccine performance for control of tick infestations on cattle. Animal Health Research Reviews 8(1): 23-28. https://doi.org/10.1017/S1466252307001193.

Gallagher MR, Kreye JK, Machtinger ET et al. 2022. Can restoration of fire-dependent ecosystems reduce ticks and tick-borne disease prevalence in the eastern United States? Ecological Applications 32(7):e2637. https://doi.org/10.1002/eap.2637.

Gilliam ME, Rechkemmer WT, McCravy KW et al. 2018. The influence of prescribed fire, habitat, and weather on *Amblyomma americanum* (Ixodida: *Ixodidae*) in West-Central Illinois, USA. Insects 9(2):36. https://doi.org/10.3390/insects9020036.

Gleim ER, Conner LM, Berghaus RD et al. 2014. The phenology of ticks and the effects of long-term prescribed burning on tick population dynamics in southwestern Georgia and northwestern Florida. PLoS One 9(11):e112174. https://doi.org/10.1371/journal.pone.0112174.

Grebner D, Bettinger P, Siry J. 2013. Common forestry practices. Introduction to Forestry and Natural Resources; Grebner, DL, Bettinger, P., Siry, JP, Eds:255-285.

Guizzo MG, Budachetri K, Adegoke A et al. 2022. *Rickettsia parkeri* infection modulates the sialome and ovariome of the Gulf coast tick, *Amblyomma maculatum*. Frontiers in Microbiology 13:1023980. https://doi.org/10.3389/fmicb.2022.1023980.

Hartman GW, Heumann B. 2003. Prescribed fire effects in the Ozarks of Missouri: the Chilton Creek Project 1996-2001. Proceedings of the second international wildland fire ecology and fire management congress:16-22. https://doi.org/ams.confex.com/ams/FI.

Hecht JA, Allerdice ME, Dykstra EA et al. 2019. Multistate survey of American dog ticks (*Dermacentor variabilis*) for *Rickettsia* species. Vector-Borne and Zoonotic Diseases 19(9):652-657. https://doi.org/10.1089/vbz.2018.2415.

Higuita NIA, Franco-Paredes C, Henao-Martínez AF. 2021. The expanding spectrum of disease caused by the lone star tick, *Amblyomma americanum*. Le Infezioni in Medicina 29(3):378. https://doi.org/10.53854/liim-2903-8.

Hoch A, Semtner PJ, Barker RW et al. 1972. Preliminary observations on controlled burning for lone star tick (Acarina: *Ixodidae*) control in woodlots. Journal of Medical Entomology 9(5):446-451. https://doi.org/10.1093/jmedent/9.5.446.

Hroobi A, Boorgula GD, Gordon D et al. 2021. Diversity and seasonality of host-seeking ticks in a periurban environment in the Central Midwest (USA). PLoS One 16(4):e0250272. https://doi.org/10.1371/journal.pone.0250272.

Hutchinson TF, Boerner RE, Sutherland S et al. 2005. Prescribed fire effects on the herbaceous layer of mixed-oak forests. Canadian Journal of Forest Research 35(4):877-890. https://doi.org/10.1139/x04-189.

Koch H, McNew R. 1981. Comparative catches of field populations of lone star ticks (*Amblyomma americanum*) by CO2 (carbon dioxide) emitting dry-ice, dry-chemical, and animal-baited devices (Oklahoma). Annals of the Entomological Society of America (USA). https://doi.org/10.1603/ME10275.

Marshall DS, Poh KC, Reichard MV et al. 2025. Spatial and temporal activity patterns of *Amblyomma americanum*. Parasites & Vectors 18(1):12. https://doi.org/10.1186/s13071-025-06661-x.

McCluskey B. 2018. Record of decision for the environmental impact statement for the Cattle Fever Eradication Program.

Molaei G, Eisen LM, Price KJ et al. 2022. Range expansion of native and invasive ticks: a looming public health threat. The Journal of Infectious Diseases 226(3):370-373. https://doi.org/10.1093/infdis/jiac249.

Ng'eno E, Alkishe A, Romero-Alvarez D et al. 2024. Phenology of five tick species in the central Great Plains. Plos one 19(5):e0302689. https://doi.org/10.1371/journal.pone.0302689.

Olds C. 2025. Ticks in Kansas. https://doi.org/bookstore.ksre.ksu.edu/pubs/ticks-in-kansas_MF2653.

Padgett K, Casher L, Stephens S et al. 2009. Effect of prescribed fire for tick control in California chaparral. Journal of Medical Entomology 46(5):1138-1145. https://doi.org/10.1603/033.046.0522.

Petry WK, Foré SA, Fielden LJ et al. 2010. A quantitative comparison of two sample methods for collecting *Amblyomma americanum* and *Dermacentor variabilis* (Acari: *Ixodidae*) in Missouri. Experimental and Applied Acarology 52:427-438. https://doi.org/10.1007/s10493-010-9373-9.

Polito VJ, Baum KA, Payton ME et al. 2013. Tick abundance and levels of infestation on cattle in response to patch burning. Rangeland ecology & management 66(5):545-552. https://doi.org/10.2111/REM-D-12-00172.1.

Raghavan RK, Peterson AT, Cobos ME et al. 2019. Current and future distribution of the lone star tick, *Amblyomma americanum* (L.)(Acari: *Ixodidae*) in North America. PloS one 14(1):e0209082. https://doi.org/10.1371/journal.pone.0209082.

Semtner PJ, Howell D, Hair JA. 1971. The ecology and behavior of the lone star tick (Acarina: *Ixodidae*) I. The relationship between vegetative habitat type and tick abundance and distribution in Cherokee County, Oklahoma. Journal of Medical Entomology 8(3):329-335. https://doi.org/10.1093/jmedent/8.3.329.

Stromdahl E, Hickling G. 2012. Beyond Lyme: Aetiology of tick-borne human diseases with emphasis on the South-Eastern United States. Zoonoses and public health 59:48-64. https://doi.org/10.1111/j.1863-2378.2012.01475.x.

Willis D, Carter R, Murdock C et al. 2012. Relationship between habitat type, fire frequency, and *Amblyomma americanum* populations in east-central Alabama. Journal of Vector Ecology 37(2):373-381. https://doi.org/10.1111/j.1948-7134.2012.00241.x.

Zimmerman RH, McWherter GR, Bloemer SR. 1987. Role of small mammals in population dynamics and dissemination of *Amblyomma americanum* and *Dermacentor variabilis* (Acari: *Ixodidae*) at Land Between the Lakes, Tennessee. Journal of Medical Entomology 24(3):370-375. https://doi.org/10.1093/jmedent/24.3.370.

Chapter 4 - Presence of microorganisms in field-collected *Amblyomma americanum* and the influence of prescribed burning

Abstract

The lone star tick, *Amblyomma americanum*, is an important vector of several pathogenic and non-pathogenic microorganisms. In the Kansas Flint Hills, prescribed burning is a common practice used to maintain tallgrass prairie and manage tick abundance. However, its influence on the microorganisms associated with ticks remains unclear. To address this question, a total of 330 adult *A. americanum* collected from unburned and seasonally burned pastures (spring, summer, and fall) were screened for *Ehrlichia chaffeensis, Ehrlichia ewingii, Francisella tularensis, Rickettsia spp.,* and *Rickettsia rickettsii* using two optimized multiplex real-time PCR assays. The overall presence was 36.67% for *Rickettsia* spp., 8.33% for *E. chaffeensis*, and 5.28% for *E. ewingii*, while *R. rickettsii* and *F. tularensis* were not detected. The high detection rate of *Rickettsia* spp. This likely reflects the presence of *R. amblyommatis*, a common endosymbiont of A. americanum, which has been linked to mild spotted fever rickettsiosis in humans. Although long-term prescribed burning reduced tick abundance, it did not alter the presence of microorganisms among ticks. Co-occurrence analysis revealed that *E. chaffeensis* and *E. ewingii* were detected together more often than expected by chance (p = 0.0007), suggesting potential interactions between these pathogens within the ticks. Taken together, these results indicate that while prescribed burning remains an effective method for reducing tick numbers, it does not appear to significantly affect the presence of microorganisms carried by *A. americanum* in the Kansas Flint Hills.

Introduction

Cases of rickettsial and ehrlichial diseases in humans and animals have increased in the United States in recent years, largely due to the rapid expansion of *Amblyomma americanum* into new regions (Goddard and Varela-Stokes 2009, Small and Brennan 2021). The aggressive and non-specific feeding behavior of this tick makes it one of the most problematic and economically important species in the country (Goddard and Varela-Stokes 2009). Because *A. americanum* feeds on a wide range of hosts, including white-tailed deer, other mammals, and humans, it plays a major role in the transmission and spread of tick-borne pathogens (Nicholson et al. 2019, McClung et al. 2023). In the Kansas Flint Hills, *A. americanum* is the dominant tick species, raising concerns about pathogen transmission to humans and animals (Ng'eno et al. 2024).

Amblyomma americanum serves as a vector of multiple pathogenic and non-pathogenic bacteria. *Ehrlichia chaffeensis* and *Ehrlichia ewingii* are intracellular bacteria that infect white blood cells, causing human monocytic ehrlichiosis (HME) and human ewingii ehrlichiosis, respectively (Goddard and Varela-Stokes 2009, Wright et al. 2014). These pathogens are maintained in nature through cycles involving white-tailed deer and other wild mammals as reservoirs (Goddard and Varela-Stokes 2009). *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever, can be acquired, maintained, and transmitted by *A. americanum* under laboratory conditions, although detections in field studies remain rare, suggesting this tick is an infrequent vector of the bacterium (Goddard and Varela-Stokes 2009, Levin et al. 2017). *Rickettsia amblyommatis*, a common endosymbiont in *A. americanum,* is likewise associated with this tick. While it is generally considered nonpathogenic, it has been linked to mild cases of spotted fever rickettsiosis in humans (Apperson et al. 2008, Goddard and Varela-Stokes 2009, McClung et al. 2023). Additionally, *A. americanum* can carry *Francisella tularensis*, the causative agent of tularemia, which infects a wide range of mammals; however, its prevalence in this tick species remains low (Mani et al. 2015, Zellner and Huntley 2019).

Prescribed burning is commonly used in the Kansas Flint Hills to maintain native tallgrass prairie and improve forage quality for grazing cattle. The previous chapter showed that long-term prescribed fire reduces tick abundance in burned pastures, likely due to changes in vegetation structure, microclimate, and host movement. What remains unclear is how prescribed burning affects the presence of microorganisms associated with *A. americanum*. Previous studies from other regions have reported mixed results depending on ecosystem type, burn frequency, and tick species (Gleim et al. 2019). Therefore, in this study, adult *A. americanum* collected from unburned and seasonally burned sites (spring, summer, and fall) were tested for *E. chaffeensis, E. ewingii, F. tularensis, Rickettsia* spp.*, and R. rickettsii* using two optimized multiplex real-time PCR assays to determine whether long-term prescribed burning influences the presence of these microorganisms.

Materials and Methods

Study areas, tick collection, and identification

The study area, tick collection methods (cloth dragging and dry ice baiting), and tick identification have been previously described in Chapters 2 and 3.

DNA extraction

A total of 55 adult *A. americanum* ticks were selected from each of the following study sections: Unburned-Control Area 1, Unburned-Control Area 2, Unburned-Control Area 3, Spring, Summer, and Fall burned pastures. Selection was based on including at least one tick per sampling date to capture a representative overview of microorganism presence across the adult activity period.

DNA was extracted individually from each adult tick. First, specimens were cut into small pieces using sterile blades, and nucleic acids were extracted with the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD, USA). Tick fragments were homogenized in 180 µL of lysis buffer with 20 µL of proteinase K using the Fisherbrand™ Bead Mill 4 Mini Homogenizer (Fisher Scientific, Waltham, MA, USA) at its highest speed (5 m/s) for 60 s with steel beads. Homogenates were incubated overnight at 56 °C, and DNA was then extracted following the manufacturer's protocol. DNA concentration was measured using an Implen NanoPhotometer (Implen, Westlake Village, CA, USA). Samples were stored at -20 °C until further testing.

Positive control design

An artificial positive control was designed using gene regions targeted by the primers for *E. chaffeensis (16S rRNA)*, *E. ewingii (16S rRNA)*, *Francisella tularensis (23 kDa protein)*, *R. rickettsii (hypothetical protein)*, and *A. americanum (MIF gene).* Each fragment included the primer-binding sites plus 10 nucleotides at both ends. All the sequences were synthesized and inserted into a pUC57 plasmid at the designated restriction site (GenScript Biotech, Piscataway, NJ, USA) (Figure 4.1). The plasmid was cloned into *Escherichia coli* DH5α competent cells (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions.

A separate positive control for *Rickettsia* spp. was produced in-house. End-point PCR was performed using Rickettsia-positive DNA with primers targeting the *citrate synthase gene (gltA).* Amplicon size was confirmed by agarose gel electrophoresis with a 100-bp ladder and SYBR Green staining. The PCR product was purified and cloned into the pCR II-Blunt-TOPO vector using the Zero Blunt TOPO PCR Cloning Kit (Thermo Fisher Scientific, Waltham, MA, USA). The plasmid was then transformed into OneShot TOP10 *E. coli* competent cells.

Both plasmids were purified with the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific, Waltham, MA, USA). DNA concentrations were determined using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Multiplex qPCR assays were conducted with 2 μL of a plasmid mixture standardized to 5 ng/μL.

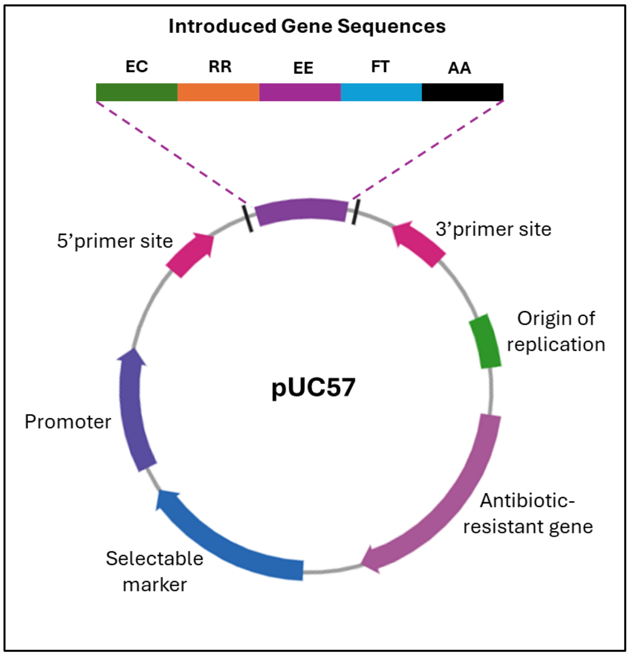


Figure 4.1. Plasmid map of the designed positive control for multiplex real-time PCR assays.

The plasmid contains fragments of target genes from *E. chaffeensis* (EC), *E. ewingii* (EE), *F. tularensis* (FT), *R. rickettsii* (RR), and *A. americanum* (AA), inserted into a pUC57 vector.

Optimization of a multiplex real-time PCR assay

Two multiplex real-time PCR assays were developed to detect five targeted microorganisms: *E. chaffeensis (16S rRNA)*, *E. ewingii (16S rRNA)*, *F. tularensis (23 kDa protein)*, *Rickettsia* spp. *(gltA gene), and R. rickettsii (hypothetical protein)*, along with an internal control gene from *A. americanum (macrophage migration inhibitory factor – MIF gene).* The primers and probes used in this study were obtained from previously published sources (Table 4.1). To confirm their specificity, the sequences were evaluated using Primer-BLAST and BLASTn (NCBI) to verify alignment with the targeted microorganism genes.

Table 4.1. Primers and probes used in the multiplex qPCR assays for detecting microorganisms associated with *A. americanum*.

Microorganism	**Target Gene**	**Primer & Probe Name**	**Sequence**	**Reference**
E. chaffeensis	***16S rRNA gene***	ECH_16srRNA_F	GCGGCAAGCCTAACACATG	(Loftis et al. 2003)
		ECH_16srRNA_R	CCCGTCTGCCACTAACAATTATT	
		ECH_16srRNA_Probe	/HEX/AGTCGAACG/ZEN/GACAATTGCTTATAACCTTTTGGT/IABkFQ/	
E. ewingii	***16S rRNA gene***	ECH_16srRNA_F	GCGGCAAGCCTAACACATG	(Gaines et al. 2014)
		EEW_16sRNA_R	CCCGTCTGCCACTAACAACTATC	
		EEW_16sRNA_Probe	/6-FAM/AGTCGAACG/ZEN/AACAATTCCTAAATAGTCTCTGAC/IABkFQ/	
F. tularensis	***23kDa protein***	FTU_23kDA_F	TGAGATGATAACAAGACAACAGGTAACA	(Versage et al. 2003)
		FTU_23kDA_R	GGATGAGATCCTATACATGCAGTAGG	
		FTU_23kDA_Probe	/TAMRA/TCAGTTCTCACATGAATGGTCTCGCCA/BHQ_2/	
***Rickettsia* spp.**	***gltA gene***	Rsp_gltA_F	GAGAGAAAATTATATCCAAATGTTGAT	Dr. Park's lab
		Rsp_gltA_R	AGGGTCTTCGTYCATTTCTT	
		Rsp_gltA_Pobe	/FAM/CATTGTGCCATCCAGCCTACGGT/3BHQ1/	
R. rickettsii	***Hypothetical protein***	RRI_HP_F	AAATCAACGGAAGAGCAAAAC	(Kato et al. 2013)
		RRI_HP_R	CCCTCCACTACCTGCATCAT	
		RRI_HP_Probe	/TAMRA/TCCTCTCCAATCAGCGATTC/BHQ_2/	
A. americanum	***MIF gene***	AA_MIF_F	CGAATCGTCTCTGCGTCTTT	(Ross et al. 2022)
		AA_MIF_R	TTTGCAGCGTTGAGAAAGTATG	
		AA_MIF_Probe	/5Cy5/TGAGTGCGA/TAO/TTTCCGTACAGAGCA/3IAbRQSp/	

Single-end-point PCR was first performed with each primer set to confirm amplification of the specific target under the same thermal conditions. An annealing temperature of 60 °C was found to be optimal and was subsequently applied in multiplex real-time PCR assays. To optimize probe concentration, multiple real-time PCRs were conducted using four final probe concentrations (50 nM, 100 nM, 200 nM, and 400 nM) as well as different combinations of these concentrations (Table 4.2). Each reaction was performed in triplicate, using the positive controls as templates and two non-template controls (water). The optimal probe concentration was defined as the condition under which all targets amplified successfully and produced the lowest cycle threshold (Ct) values.

Table 4.2. Final probe concentrations used in multiplex real-time PCR assays.
Reaction 1 targeted *E. chaffeensis*, *E. ewingii*, *F. tularensis*, and *A. americanum* (internal control), while Reaction 2 targeted *Rickettsia* spp., *R. rickettsii*, and *A. americanum* (internal control).

Selected Probe Concentration			
Reaction 1		**Reaction 2**	
E. chaffeensis	50 (µM)	***Rickettsia* spp.**	50 (µM)
E. ewingii	50 (µM)	***R. rickettsii***	200 (µM)
F. tulerensis	100 (µM)	***A. americanum***	200 (µM)
A. americanum	100 (µM)		

Screening of tick samples

Field-collected tick DNA samples were analyzed using two optimized multiplex real-time PCR assays. Each reaction was performed in a 20 µL final volume consisting of 10 µL TaqMan Multiplex Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µL of primer mix (10 µM of each primer set), the appropriate probe concentrations (Table 4.2), 5 µL of DNA template, and nuclease-free water. Amplification was carried out with the following thermal profile: an initial denaturation at 95 °C for 20 seconds, followed by 40 cycles of denaturation at

95 °C for 1 second and annealing/extension at 60 °C for 35 seconds. Reactions were run on a QuantStudio 7 Pro Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). Each run included positive controls (constructed plasmids) and a non-template control (nuclease-free water).

Single-end-point PCR assays were performed using six randomly selected *E. chaffensis* positive samples, six *E. ewingii* positive samples, and 13 *Rickettsia* spp. positive samples. The resulting amplicons were purified using the DNA Clean Concentrator kit (Zymo Research, Irvine, CA, USA) and submitted for Sanger sequencing at Genewiz (Azenta, South Plainfield, NJ, USA). The obtained sequences were compared against all the available sequences published in GenBank for confirmation.

Statistical analysis

Data were analyzed at the individual tick level, where detection status was 0 (no microorganism detected) and 1 (microorganism detected). A logistic regression model was used to evaluate whether long-term prescribed fire influenced the presence of targeted microorganisms in *A. americanum.* The model includes treatments (unburned, spring, summer, and fall burns) and tick sex (male and female) as factors. Separate models were run for each microorganism, *E. chaffeensis*, *E. ewingii, and Rickettsia* spp. Because *F. tularensis* and *R. rickettsii* were not detected in any samples, they were excluded from the analysis. Odds ratios with 95% confidence intervals were calculated to compare the likelihood of microorganism presence among treatments and sexes, and pairwise comparisons were used to identify differences among treatments.

The presence of each microorganism was summarized by treatment and visualized with a bar plot. The number of microorganisms per tick (none, one, two, or three) was also summarized to observe co-detections. To determine whether the microorganisms occurred by chance or showed patterns of association, I compared the observed number of ticks carrying none, one, two, or three microorganisms with the number expected if each microorganism were acquired independently. Expected values were generated using a bootstrap simulation with 10,000 iterations. The observed and expected distributions were then compared with a G-test. Additionally, pairwise associations between microorganisms were assessed using Fisher's exact test to estimate odds ratios and confidence intervals. These analyses determined whether ticks carrying one microorganism were more or less likely to also carry another. A network diagram was created to visualize significant associations among the detected microorganisms. All analyses and figures were produced in RStudio (version 4.5.1).

Results

Microorganism detected in burned and unburned pastures

The presence of microorganisms detected in adults *A. americanum* varied across prescribed burning treatments and the unburned control site (Table 4.3; Figure 4.2). For *E. chaffeensis*, presence was lowest in summer-burned sites (3.64%) and highest in those burned in fall (16.36%), with intermediate values in unburned (9.09%) and spring-burned (7.27%) areas. The presence of *E. ewingii* followed a similar pattern, with the lowest detection in summer-burned areas (1.82%) and the highest in fall-burned areas (10.91%). While unburned and spring-burned areas had the same presence of 5.45%. *Rickettsia* spp. was detected at high levels across all treatments, ranging from 33.94% in unburned areas to 41.82% in both summer- and fall-burned areas. In contrast, none of the samples tested positive for *R. rickettsii* and *F. tularensis*.

Table 4.3. Presence of *E. chaffeensis*, *E. ewingii*, and *Rickettsia* spp. in *A. americanum* collected from unburned and seasonally burned sites.

Microorganism	Treatment	Positives/Total	Detection (%)	95% CI

E. chaffeensis	Unburned	15/165	9.09	5.18-14.55
	Spring	4/55	7.27	2.02-17.59
	Summer	2/55	3.64	0.44-12.53
	Fall	9/55	16.36	7.77-28.80
E. ewingii	Unburned	9/165	5.45	2.52-10.10
	Spring	3/55	5.45	1.14-15.12
	Summer	1/55	1.82	0.05-9.72
	Fall	6/55	10.91	4.11-22.25
***Rickettsia* spp.**	Unburned	56/165	33.94	26.76-41.71
	Spring	19/55	34.55	22.24-48.58
	Summer	23/55	41.82	28.65-55.89
	Fall	23/55	41.82	28.65-55.89

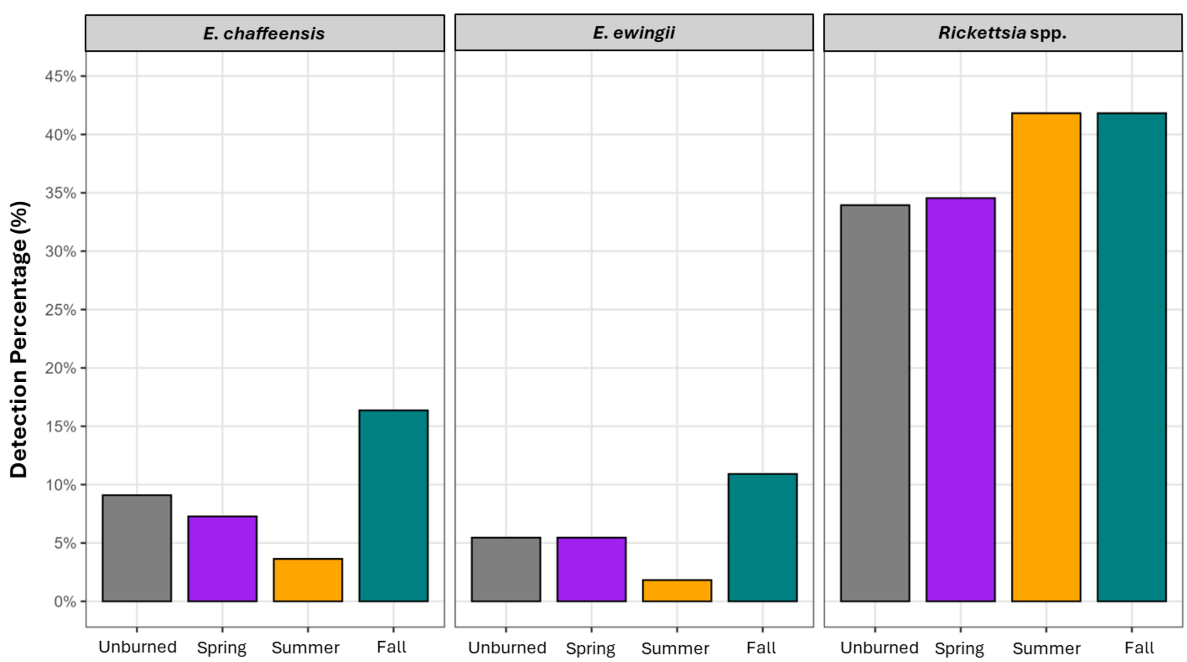


Figure 4.2. Comparison of microorganism presence in *A. americanum* collected from unburned, spring-, summer-, and fall-burned sites.

Impact of the prescribed burning on the presence of microorganisms

Logistic regression analysis was used to assess whether the presence of microorganisms differed between seasonal prescribed fire treatments and unburned areas (Table 4.4). For *E. ewingii*, ticks collected from fall-burned sites had the highest likelihood of detection (OR = 8.35, 95% CI: 2.05-525.9), while those from spring and summer burned pastures did not differ significantly from unburned areas. A similar trend was observed for *E. chaffeensis*, where the odds of detection were greater in fall-burned sites (OR = 7.07, 95% CI: 2.23-17.3); however, the wide confidence intervals and p-value suggested no significant effect. For *Rickettsia* spp., detection tends to be higher in all burned treatments compared to unburned areas, but none of the comparisons were statistically significant. Pairwise comparisons among treatments also revealed no significant differences for any of the three microorganisms analyzed. Overall, prescribed fire did not significantly influence the presence of microorganisms in *A. americanum.*

Table 4.4. Logistic regression results for the presence of microorganisms in *A. americanum* across seasonal prescribed fire treatments compared with unburned sites.

Microorganism	Seasonal Fire Treatments vs Unburned	Odds Ratios	95% CI (Lower – Upper)	p – value
E. ewingii	Spring vs Unburned	2.72	1.30 – 46.2	1
	Summer vs Unburned	1.38	1.04 – 13.4	0.286
	Fall vs Unburned	8.35	2.05 – 525.9	0.172
E. chaffeensis	Spring vs Unburned	2.19	1.28 – 11.8	0.678
	Summer vs Unburned	1.46	1.09 – 5.50	0.205
	Fall vs Unburned	7.07	2.23 – 117.0	0.139
***Rickettsia* spp.**	Spring vs Unburned	2.80	1.63 – 8.79	0.938
	Summer vs Unburned	4.07	1.96 – 18.6	0.363

	Fall vs Unburned	4.07	1.96 – 18.6	0.363

Differences in microorganism presence between female and male ticks

In addition to seasonal burn treatments, the presence of microorganisms was also compared between male and female ticks (Table 4.5). Males showed a higher likelihood of detection for *E. ewingii* (OR = 4.53), *E. chaffeensis* (OR = 2.95), and *Rickettsia* spp. (OR = 3.00) than females. While these results indicate that males may carry microorganisms more often than females, the differences were not statistically significant.

Table 4.5. Logistic regression results for the presence of microorganisms in *A. americanum* males and females.

Microorganism	Sex	Odds Ratio	95% CI (Lower – Upper)	p-value
E. ewingii	Males vs Females	4.53	1.80 – 48.9	0.392
E. chaffeensis	Males vs Females	2.95	1.66 – 9.94	0.841
***Rickettsia* spp.**	Males vs Females	3.00	2.01 – 5.64	0.689

Ticks carrying multiple microorganisms

Around 60% of the ticks tested negative for all microorganisms, while 38.8% carried a single microorganism. A smaller number carried multiple microorganisms, with 3.6% testing positive for two and 1.8% for three (Figure 4.3). Overall, most ticks were either uninfected or carried only one microorganism, although a few individual ticks harbored multiple microorganisms at the same time.

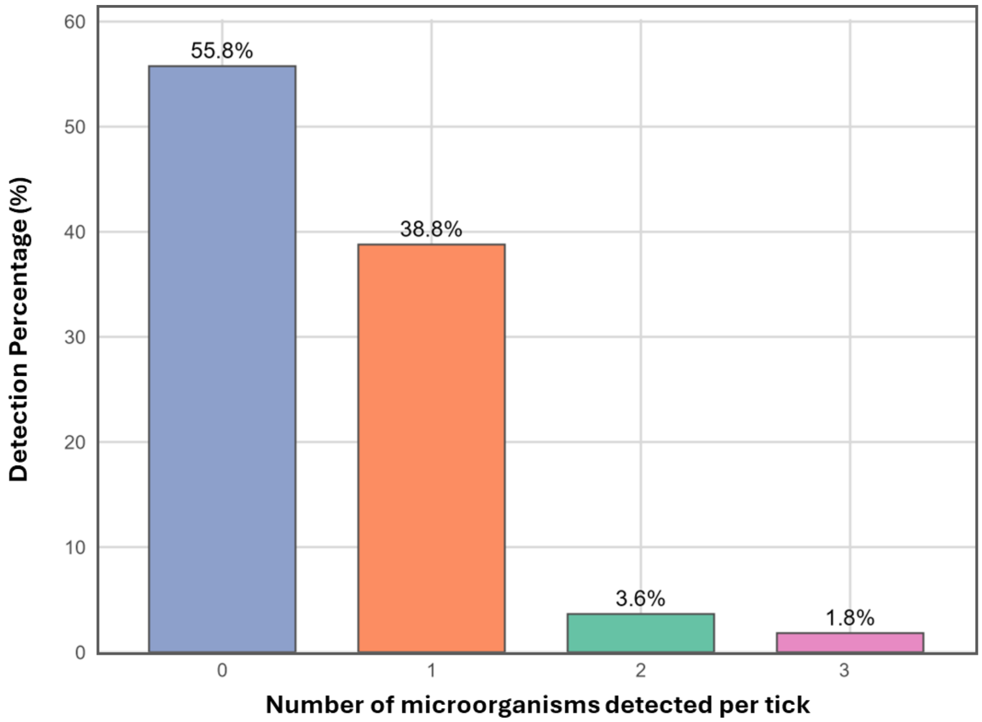


Figure 4.3. *A. americanum* carrying multiple microorganisms.

The distribution of ticks carrying none, one, two, and three microorganisms differed significantly from what would be expected by random change (bootstrap G-test, p = 0.0007), indicating that some microorganisms tend to occur more often than predicted. Most ticks were either uninfected (n = 184) or carried a single microorganism (n = 128), which were close to the expected values. Double detections were less common than predicted (12 observed vs 18 expected), while triple detections occurred more often than expected (6 observed vs <1 predicted) (Figure 4.4). Pairwise analysis showed a strong positive association between *E. ewingii* and *E. chaffeensis* (odds ratio = 27.2, 95% CI: 8.7–92.5, p < 0.001) (Table 4.6). No significant associations were found between *Rickettsia* spp. and either *Ehrlichia* spp. The network diagram represents these results, showing a strong connection between the two *Ehrlichia* species and no connections with *Rickettsia* spp. (Figure 4.5).

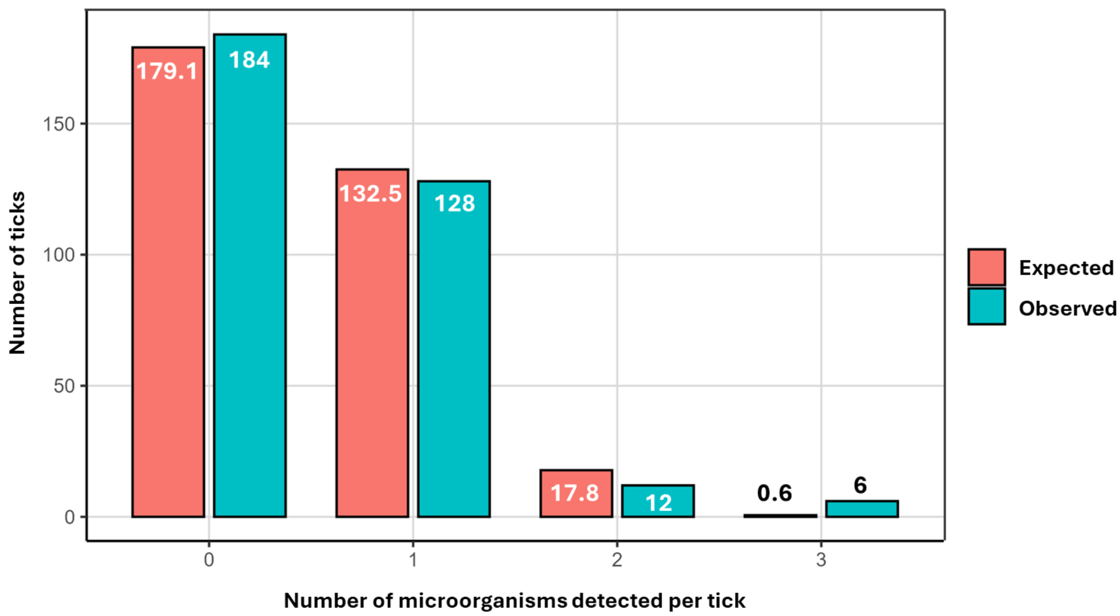


Figure 4.4. Comparison between the observed and expected number of ticks carrying 0, 1, 2, or 3 microorganisms.

Bars in blue represent the observed counts, and bars in red show the counts expected under a random distribution model.

Table 4.6. Pairwise associations between microorganisms detected in *A. americanum.*

Microorganism A	Microorganism B	Odds ratio	95% CI	p-value
E. ewingii	*E. chaffeensis*	27.2	8.7 – 92.5	<0.001
E. ewingii	*Rickettsia* spp.	1.0	0.3 – 2.9	1.0
E. chaffeensis	*Rickettsia* spp.	1.0	0.4 – 2.3	1.0

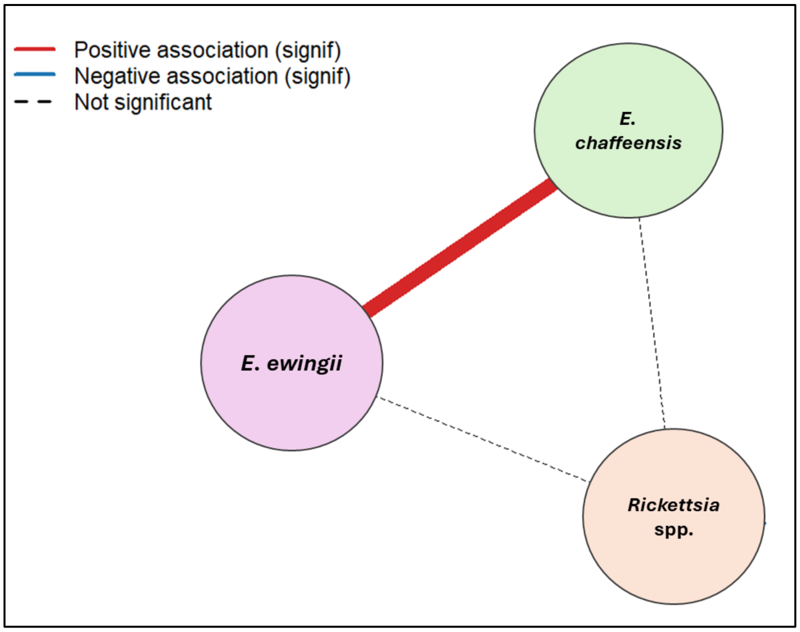


Figure 4.5. Network diagram showing associations among microorganisms in *A. americanum*.

A strong positive association was found between *E. ewingii* and *E. chaffeensis* (red line), while *Rickettsia* spp. showed no significant links (dashed lines).

Discussion

This study provides important findings on the presence of microorganisms associated with *A. americanum* in an understudied ecosystem, the tallgrass prairie of the Kansas Flint Hills. Among the 330 adult *A. americanum* tested, *Rickettsia* spp. were detected in 36.67% of samples, a value consistent with previous reports for this tick species across the United States (11.8–37.3%) (Mixson et al. 2006, Small and Brennan 2021, Noden et al. 2022, McClung et al. 2023). The relatively high detection of *Rickettsia* spp. observed here likely reflects the presence of *R. amblyommatis*, an endosymbiont maintained in *A. americanum* populations through both transovarial and transstadial transmission, resulting in higher detection rates (Small and Brennan 2021, Zając et al. 2023). Previous studies have reported that between 23.8 and 41.2% of adult *A.*

americanum carry *R. amblyommatis* (Mixson et al. 2006, Berrada et al. 2011, Karpathy et al. 2016, McClung et al. 2023).

For *Ehrlichia* spp., presence was lower than for *Rickettsia* spp, with *E. chaffeensis* detected in 8.33% and *E. ewingii* in 5.28% of ticks. These values fall within the range reported in previous studies (1%-17.3%) (Mixson et al. 2006, Sirigireddy et al. 2006, Small and Brennan 2021, McClung et al. 2023). Unlike *Rickettsia* spp., *Ehrlichia* spp. are transmitted only transstadial and must be acquired from an infected vertebrate host, such as white-tailed deer or dogs (Blanton et al. 2014, Small and Brennan 2021). As a result, the presence of these pathogens depends on the availability of reservoir hosts in the area. This difference in transmission mode likely explains why *Rickettsia* spp. were more frequently detected than *Ehrlichia* spp. in this study.

The difference in *Ehrlichia* presence between unburned and fall-burned sites may be influenced by changes in habitat composition and wildlife movement. Fall-burned areas are more open, with less tree cover, which likely facilitates the movement of reservoir hosts such as white-tailed deer (Walls et al. 1998). In contrast, unburned areas are surrounded by denser tree cover that may limit host activity and reduce the opportunity for pathogen transmission. Host use of the landscape is important because wildlife movement has been shown to influence the distribution of ticks and the pathogens they carry. For instance, previous studies have found that wildlife diversity and movement were linked to higher *Borrelia burgdorferi* presence in *Ixodes pacificus*, and that habitat connectivity and fragmentation influenced both tick abundance and infection rates (Shaw et al. 2024). These findings indicate that the relationship between habitat structure and host movement could help explain the differences in pathogen presence observed between unburned and fall-burned sites in this study.

As shown in Chapter 3, long-term prescribed burning significantly reduced tick abundance in the Kansas Flint Hills; however, the presence of the screened microorganisms did not differ between burned and unburned areas. Previous studies have also examined our question regarding the association between fire and microbial presence. In California, wildfire studies found no consistent reduction in *Borrelia* spp. presence due to low overall infection rate (MacDonald et al. 2018). In southwestern Georgia and northwestern Florida, the long-term prescribed fire had no effect on bacterial pathogen presence in ticks collected from mixed pine ecosystems (Gleim et al. 2019). Interestingly, this study reported a lower detection of *Rickettsia* spp. in burned sites compared to unburned sites, whereas our data showed no difference across treatments. Overall, these results suggest that while long-term prescribed burning effectively lowers tick abundance, its influence on the presence of microorganisms is limited and may vary depending on the ecosystem and microbial species involved.

Microorganism presence was slightly higher in male ticks than in females, but the difference was not statistically significant. Comparable results have been reported for *Rickettsia* spp., where presence did not differ between males and females (McClung et al. 2023). This suggests that both male and female *A. americanum* contribute equally to the maintenance and transmission of microorganisms. We also detected ticks carrying multiple microorganisms, a pattern reported in previous studies documenting co-detections involving *E. chaffeensis* and *E. ewingii*, or combinations of *Ehrlichia* and *Rickettsia* species in ticks and vertebrate hosts (Kordick et al. 1999, Sirigireddy et al. 2006, Beall et al. 2012, Small and Brennan 2021, McClung et al. 2023). The occurrence of multiple microorganisms within individual ticks suggests the potential for simultaneous transmission, which could increase disease severity and present a greater risk to human and animal health.

Conclusion

This study contributes to understanding the relationship between *A. americanum* and the microorganisms it carries within the tallgrass prairie ecosystem. Long-term prescribed fire was effective in reducing tick numbers but did not alter the presence of microorganisms detected. *Rickettsia* spp. was the most common microorganism detected, likely representing *R. amblyommatis*, an endosymbiont commonly found in *A. americanum*. This species has been occasionally associated with mild cases of spotted fever rickettsiosis in humans. The presence of multiple microorganisms within individual ticks suggests that co-infections can occur, increasing the possibility of simultaneous transmission and more severe disease outcomes. Overall, these findings underscore the importance of conducting research in both vector and pathogen ecology when developing integrated management strategies to control ticks and tick-borne diseases.

References

Apperson CS, Engber B, Nicholson WL et al. 2008. Tick-borne diseases in North Carolina: is "*Rickettsia amblyommii*" a possible cause of rickettsiosis reported as Rocky Mountain spotted fever? Vector-Borne and Zoonotic Diseases 8(5):597-606. https://doi.org/10.3201/eid1705.101530.

Beall MJ, Alleman AR, Breitschwerdt EB et al. 2012. Seroprevalence of *Ehrlichia canis, Ehrlichia chaffeensis* and *Ehrlichia ewingii* in dogs in North America. Parasites & vectors 5(1):29. https://doi.org/10.1186/1756-3305-5-29.

Berrada ZL, Goethert HK, Cunningham J et al. 2011. *Rickettsia rickettsii* (Rickettsiales: *Rickettsiaceae*) in *Amblyomma americanum* (Acari: *Ixodidae*) from Kansas. Journal of Medical Entomology 48(2):461-467. https://doi.org/10.1603/me10130.

Blanton LS, Walker DH, Bouyer DH. 2014. Rickettsiae and ehrlichiae within a city park: is the urban dweller at risk? Vector-Borne and Zoonotic Diseases 14(2):168-170. https://doi.org/10.1089/vbz.2013.1473.

Gaines DN, Operario DJ, Stroup S et al. 2014. Ehrlichia and spotted fever group Rickettsiae surveillance in *Amblyomma americanum* in Virginia through use of a novel six-plex real-time PCR assay. Vector-Borne and Zoonotic Diseases 14(5):307-316. https://doi.org/10.1089/vbz.2013.1509.

Gleim ER, Zemtsova GE, Berghaus RD et al. 2019. Frequent prescribed fires can reduce risk of tick-borne diseases. Scientific Reports 9(1):9974. https://doi.org/10.1038/s41598-019-46377-4.

Goddard J, Varela-Stokes AS. 2009. Role of the lone star tick, *Amblyomma americanum* (L.), in human and animal diseases. Veterinary Parasitology 160(1-2):1-12. https://doi.org/10.1016/j.vetpar.2008.10.089.

Karpathy SE, Slater KS, Goldsmith CS et al. 2016. *Rickettsia amblyommatis* sp. nov., a spotted fever group *Rickettsia* associated with multiple species of *Amblyomma* ticks in North, Central and South America. International Journal of Systematic and Evolutionary Microbiology 66(12):5236-5243. https://doi.org/10.1099/ijsem.0.001502.

Kato CY, Chung IH, Robinson LK et al. 2013. Assessment of real-time PCR assay for detection of *Rickettsia* spp. and *Rickettsia rickettsii* in banked clinical samples. Journal of Clinical Microbiology 51(1):314-317. https://doi.org/10.1128/JCM.01723-12.

Kordick SK, Breitschwerdt E, Hegarty B et al. 1999. Coinfection with multiple tick-borne pathogens in a Walker Hound kennel in North Carolina. Journal of Clinical Microbiology 37(8):2631-2638. https://doi.org/10.1128/JCM.37.8.2631-2638.1999.

Levin ML, Zemtsova GE, Killmaster LF et al. 2017. Vector competence of *Amblyomma americanum* (Acari: Ixodidae) for *Rickettsia rickettsii*. Ticks and tick-borne diseases 8(4):615-622. https://doi.org/10.1016/j.ttbdis.2017.04.006.

Loftis AD, Massung RF, Levin ML. 2003. Quantitative real-time PCR assay for detection of *Ehrlichia chaffeensis*. Journal of Clinical Microbiology 41(8):3870-3872. https://doi.org/10.1128/JCM.41.8.3870-3872.2003.

MacDonald AJ, Hyon DW, McDaniels A et al. 2018. Risk of vector tick exposure initially increases, then declines through time in response to wildfire in California. Ecosphere 9(5):e02227. https://doi.org/10.1002/ecs2.2227.

Mani RJ, Metcalf JA, Clinkenbeard KD. 2015. *Amblyomma americanum* as a bridging vector for human infection with *Francisella tularensis*. PLoS One 10(6):e0130513. https://doi.org/10.1371/journal.pone.0130513.

McClung KL, Sundstrom KD, Lineberry MW et al. 2023. Seasonality of *Amblyomma americanum* (Acari: *Ixodidae*) activity and prevalence of infection with tick-borne disease agents in north central Oklahoma. Vector-Borne and Zoonotic Diseases 23(11):561-567. https://doi.org/10.1089/vbz.2023.0009.

Mixson TR, Campbell SR, Gill JS et al. 2006. Prevalence of *Ehrlichia*, *Borrelia*, and *Rickettsial* agents in *Amblyomma americanum* (Acari: *Ixodidae*) collected from nine states. Journal of Medical Entomology 43(6):1261-1268. https://doi.org/10.1603/0022-2585(2006)43[1261:poebar]2.0.co;2.

Ng'eno E, Alkishe A, Romero-Alvarez D et al. 2024. Phenology of five tick species in the central Great Plains. Plos one 19(5):e0302689. https://doi.org/10.1371/journal.pone.0302689.

Nicholson WL, Sonenshine DE, Noden BH et al. 2019. Ticks (*Ixodida*). Medical and Veterinary Entomology: 603-672. https://doi.org/10.1016/B978-012510451-7/50026-8.

Noden BH, Dubie TR, Henriquez BE et al. 2022. Seasonality of ticks and prevalence of *Rickettsiae* species in *Dermacentor variabilis* and *Amblyomma maculatum* across Oklahoma pastures. Journal of Medical Entomology 59(3):1033-1041. https://doi.org/10.1093/jme/tjac028.

Ross A-ML, Stokes JV, Cross CE et al. 2022. Multiplex TaqMan® Quantitative PCR Assays for Host-Tick-Pathogen Studies Using the Guinea Pig-Tick-*Rickettsia* System. Pathogens 11(5):594. https://doi.org/10.3390/pathogens11050594.

Shaw G, Lilly M, Mai V et al. 2024. The roles of habitat isolation, landscape connectivity and host community in tick-borne pathogen ecology. Royal Society Open Science 11(11):240837. https://doi.org/10.1098/rsos.240837.

Sirigireddy KR, Mock DC, Ganta RR. 2006. Multiplex detection of *Ehrlichia* and *Anaplasma* pathogens in vertebrate and tick hosts by real-time RT-PCR. Annals of the New York Academy of Sciences 1078(1):552-556. https://doi.org/10.1196/annals.1374.108.

Small M, Brennan RE. 2021. Detection of *Rickettsia amblyommatis* and *Ehrlichia chaffeensis* in *Amblyomma americanum* inhabiting two urban parks in Oklahoma. Vector-Borne and Zoonotic Diseases 21(5):385-387. https://doi.org/10.1089/vbz.2020.2755.

Versage JL, Severin DD, Chu MC et al. 2003. Development of a multitarget real-time TaqMan PCR assay for enhanced detection of *Francisella tularensis* in complex specimens. Journal of Clinical Microbiology 41(12):5492-5499. https://doi.org/10.1128/JCM.41.12.5492-5499.2003.

Walls JJ, Asanovich KM, Bakken JS et al. 1998. Serologic evidence of a natural infection of white-tailed deer with the agent of human granulocytic ehrlichiosis in Wisconsin and Maryland. Clinical Diagnostic Laboratory Immunology 5(6):762-765. https://doi.org/10.1128/CDLI.5.6.762-765.1998.

Wright CL, Gaff HD, Hynes WL. 2014. Prevalence of *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in *Amblyomma americanum* and *Dermacentor variabilis* collected from southeastern Virginia, 2010–2011. Ticks and tick-borne diseases 5(6):978-982. https://doi.org/10.1016/j.ttbdis.2014.07.023.

Zając Z, Obregon D, Foucault-Simonin A et al. 2023. Disparate dynamics of pathogen prevalence in *Ixodes ricinus* and *Dermacentor reticulatus* ticks occurring sympatrically in diverse habitats. Scientific Reports 13(1):10645. https://doi.org/10.1038/s41598-023-37748-z.

Zellner B, Huntley JF. 2019. Ticks and tularemia: do we know what we don't know? Frontiers in cellular and infection microbiology 9:146. https://doi.org/10.3389/fcimb.2019.00146.

Chapter 5 - Research summary

Ticks are the most common blood-feeding arthropods impacting humans and animals worldwide. After mosquitoes, they are considered the most important group of vectors transmitting pathogens. Ticks are expanding their geographic distribution in new areas, and tick-borne disease cases have also increased, generating global concern (Jongejan and Uilenberg 2004, Dantas-Torres et al. 2012). In addition to transmitting multiple pathogens, ticks harm their hosts directly by causing blood loss, irritation, and skin damage that can lead to secondary infections (Gerem Eskezia and Hadush Desta 2016, Basu and Charles 2017, Abubakar and Perera 2019). In the Central Great Plains, the lone star tick (*Amblyomma americanum*) is the most abundant species and represents approximately 97% of the specimens collected in recent studies (Peterson 2012, Ng'eno et al. 2024). This species is widely distributed across the Eastern United States, ranging from Texas and Kansas to the Atlantic coast (CDC 2019). All life stages feed on humans or animals, making the species an effective vector for several pathogens (Goddard and Varela-Stokes 2009). Tick activity usually begins in early spring and continues through fall. Field studies from Missouri have shown that the life cycle of the tick takes approximately two years to complete, which provides a useful comparison for tick populations in Kansas (Bouzek et al., 2013). As *A. americanum* continues to expand its distribution, it is necessary to develop management strategies that can reduce the impact of ticks on human and livestock health.

Traditional control methods include the use of acaricides, biological control, anti-tick vaccines, and resistant cattle breeds (Basu and Charles 2017, Perumalsamy et al. 2024). However, these approaches differ in their effectiveness, cost, and environmental safety. Prescribed burning has been considered as a non-chemical alternative to reduce tick populations. However, results from previous studies are inconsistent. Some studies have shown significant decreases in tick abundance after burning, while others found no effects of the fire on tick populations (Davidson et al. 1994a, Padgett et al. 2009, Willis et al. 2012, Polito et al. 2013, Gleim et al. 2014). These differences suggest that additional studies are needed to evaluate how factors such as burn timing, vegetation composition, and habitat characteristics could influence the effectiveness of prescribed fire as a tick control method.

In this dissertation, the seasonal abundance of *A. americanum* (Chapter 2), the presence of pathogenic and non-pathogenic microorganisms in field-collected ticks (Chapter 4), and the effect of prescribed burning as a possible method to reduce tick populations and pathogen transmission (Chapters 3 and 4) were studied. Understanding the timing of tick activity and how prescribed burning affects both tick numbers and pathogen presence is necessary for developing sustainable management strategies that support public health and livestock production.

In Chapter 2, the seasonal life cycle of *A. americanum* in the Kansas Flint Hills was characterized to better understand the population dynamics throughout the year and the environmental factors that influence it. Field observations suggest that the life cycle of this tick species takes about two years, which is similar to findings from northeast Missouri (Bouzek et al. 2013). During the first year, adults lay eggs, and larvae hatch, with host-seeking activity commonly observed from late July through October. Unfed larvae do not often survive the winter, so they must either feed and overwinter or molt into nymphs before entering a dormancy phase (Sonenshine and Levy 1971, Koch 1984, Ma et al. 2021, Foré et al. 2023). In the following spring, nymphs reemerge, becoming active from March until October or November. By late fall, engorged nymphs molt into adults, which then overwinter and complete the cycle the next year (Bouzek et al. 2013, Foré et al. 2023).

The difference in timing among life stages indicates that adults and larvae occur in separate years from nymphs, suggesting that two cohorts may coexist in the same areas. Although this pattern has not been confirmed using genetic or genomic approaches, it brings up important questions about population structure and how ticks adapt to environmental conditions. In this study, a gradual shift toward early adult and nymph emergence was observed over three sampling years, which reflects the influence of increasing environmental temperatures on tick phenology. These earlier activity periods may extend the season of exposure for both humans and animals, underscoring the importance of maintaining long-term surveillance and use of tick control methods, such as prescribed burning, to reduce future risk.

In Chapter 3, the effectiveness of sustained prescribed burning as a management strategy to reduce *A. americanum* populations in the Kansas Flint Hills was evaluated. Prescribed fire has been used in this region as a land management tool to improve forage quality and cattle productivity. At the Kansas State University Beef Stocker Unit, prescribed burns have been applied for the past five years across three pasture areas, each treated in a different season (spring, summer, and fall). Ticks were collected from all treatments to assess how the timing of the prescribed fire influenced their abundance. Burned pastures had significantly fewer ticks than the unburned control areas. Sites burned during the spring season (April) had the lowest tick numbers. These findings align with published studies, which reported that spring fires reduce leaf litter and vegetation cover, leading to drier microclimates that are less suitable for tick survival (Hoch et al. 1972, Davidson et al. 1994b, Hutchinson et al. 2005, Allan 2009). Sustained burning over several years helped maintain reduced tick populations, suggesting the application of the prescribed fire every year suppresses tick activity. Similar results were reported in other studies, where long-term prescribed fire limited tick survival (Cully 1999, Gleim et al. 2014). Overall, these results demonstrate that prescribed burning could serve as an effective, non-chemical option for integrated tick management.

In Chapter 4, the determined the impact of prescribed burning on the presence of microorganisms in *A. americanum* collected from sites under different burned and unburned conditions. The overall detection was 36.67% for *Rickettsia* spp., 8.33% for *E. chaffeensis*, and 5.28% for *E. ewingii*, while *R. rickettsii* and *F. tularensis* were not detected. The presence of *Rickettsia* spp. observed in this study was within the range previously reported for this species in the United States (11.8–37.3%) (Mixson et al. 2006, McClung et al. 2023). Similarly, the values evaluated for two *Ehrlichia* species were also found in earlier studies (1–17.3%) (Mixson et al. 2006, Sirigireddy et al. 2006, Small and Brennan 2021, Noden et al. 2022, McClung et al. 2023). Among all the tested microorganisms, *Rickettsia* spp. were the most common, likely representing *R. amblyommatis,* an endosymbiont commonly found in *A. americanum* and occasionally associated with mild spotted fever in humans (Mixson et al. 2006, Berrada et al. 2011, Karpathy et al. 2016, McClung et al. 2023). As discussed in Chapter 3, long-term prescribed burning significantly reduced tick abundance in the Kansas Flint Hills; however, the presence of tested microorganisms did not differ between burned and unburned areas. A similar study conducted in the mixed pine forests of Georgia and Florida also found no consistent reduction in pathogen presence after long-term burning (Gleim et al. 2019). In contrast to this study, where *Rickettsia* infection was lower in burned areas, our results showed detection across all treatments. Co-occurrence analyses indicated that *E. chaffeensis* and *E. ewingii* were detected together more often than expected by chance ($p = 0.0007$), suggesting potential interactions between these pathogens within the tick. In this study, several ticks also tested positive for multiple microorganisms, which is consistent with previous reports of co-infections involving *Ehrlichia* and *Rickettsia* species in both ticks and vertebrate hosts (Kordick et al. 1999, Beall et al. 2012, Small and Brennan 2021, McClung et al. 2023). These results demonstrate that although prescribed burning reduces tick abundance, its influence on pathogen presence remains unclear.

Overall, this research contributes to a better understanding of *A. americanum* ecology, pathogen dynamics, and the role of prescribed burning as a tick control mechanism in the Kansas Flint Hills.

References

Abubakar M, Perera PK. 2019. *Ticks and Tick-Borne Pathogens, BoD–Books on Demand.*

Allan BF. 2009. Influence of prescribed burns on the abundance of *Amblyomma americanum* (Acari: *Ixodidae*) in the Missouri Ozarks. Journal of Medical Entomology 46(5):1030-1036. https://doi.org/10.1603/033.046.0509.

Basu AK, Charles R. 2017. Ticks of Trinidad and Tobago: an overview.

Beall MJ, Alleman AR, Breitschwerdt EB et al. 2012. Seroprevalence of *Ehrlichia canis, Ehrlichia chaffeensis* and *Ehrlichia ewingii* in dogs in North America. Parasites & vectors 5(1):29. https://doi.org/10.1186/1756-3305-5-29.

Berrada ZL, Goethert HK, Cunningham J et al. 2011. *Rickettsia rickettsii* (*Rickettsiales: Rickettsiaceae*) in *Amblyomma americanum* (Acari: *Ixodidae*) from Kansas. Journal of medical entomology 48(2):461-467. https://doi.org/10.1603/me10130.

Bouzek D, Foré S, Bevell J et al. 2013. A conceptual model of the *Amblyomma americanum* life cycle in northeast Missouri. Journal of Vector Ecology 38(1):74-81. https://doi.org/10.1111/j.1948-7134.2013.12011.x.

Center of Diasease Control - CDC. 2019. Geographic distribution of ticks that bite humans. https://doi.org/www.cdc.gov/ticks/about/where-ticks-live.html.

Cully JF. 1999. Lone star tick abundance, fire, and bison grazing in tallgrass prairie. Rangeland Ecology & Management/Journal of Range Management Archives 52(2):139-144. https://doi.org/10.2307/4003507.

Dantas-Torres F, Chomel BB, Otranto D. 2012. Ticks and tick-borne diseases: a One Health perspective. Trends in parasitology 28(10):437-446. https://doi.org/10.1016/j.pt.2012.07.003.

Davidson WR, Siefken DA, Creekmore LH. 1994a. Seasonal and annual abundance of *Amblyomma americanum* (Acari: *Ixodidae*) in central Georgia. Journal of Medical Entomology 31(1):67-71. https://doi.org/10.1093/jmedent/31.1.72.

Davidson WR, Siefken DA, Creekmore LH. 1994b. Influence of annual and biennial prescribed burning during March on the abundance of *Amblyomma americanum* (Acari: *Ixodidae*) in central Georgia. Journal of Medical Entomology 31(1):72-81. https://doi.org/10.1093/jmedent/31.1.72.

Foré SA, Mangan MJ, Mantia AM et al. 2023. Multiple physiological cohorts comprise seasonal activity of wild *Amblyomma americanum* (Acari: *Ixodidae*) nymphs. Ticks and Tick-borne Diseases 14(2):102091. https://doi.org/10.1016/j.ttbdis.2022.102091.

Gerem Eskezia B, Hadush Desta A. 2016. Review on the impact of ticks on livestock health and productivity. J. Bio. Agr. Health 6:1-7.

Gleim ER, Conner LM, Berghaus RD et al. 2014. The phenology of ticks and the effects of long-term prescribed burning on tick population dynamics in southwestern Georgia and northwestern Florida. PLoS One 9(11):e112174. https://doi.org/10.1371/journal.pone.0112174.

Gleim ER, Zemtsova GE, Berghaus RD et al. 2019. Frequent prescribed fires can reduce risk of tick-borne diseases. Scientific Reports 9(1):9974. https://doi.org/10.1038/s41598-019-46377-4.

Goddard J, Varela-Stokes AS. 2009. Role of the lone star tick, *Amblyomma americanum* (L.), in human and animal diseases. Veterinary parasitology 160(1-2):1-12. https://doi.org/10.1016/j.vetpar.2008.10.089.

Hoch A, Semtner PJ, Barker RW et al. 1972. Preliminary observations on controlled burning for lone star tick (Acarina: *Ixodidae*) control in woodlots. Journal of Medical Entomology 9(5):446-451. https://doi.org/10.1093/jmedent/9.5.446.

Hutchinson TF, Boerner RE, Sutherland S et al. 2005. Prescribed fire effects on the herbaceous layer of mixed-oak forests. Canadian Journal of Forest Research 35(4):877-890. https://doi.org/10.1139/x04-189.

Jongejan F, Uilenberg G. 2004. The global importance of ticks. Parasitology 129(S1):S3-S14. https://doi.org/10.1017/s0031182004005967.

Karpathy SE, Slater KS, Goldsmith CS et al. 2016. *Rickettsia amblyommatis* sp. nov., a spotted fever group *Rickettsia* associated with multiple species of *Amblyomma* ticks in North, Central and South America. International Journal of Systematic and Evolutionary Microbiology 66(12):5236-5243. https://doi.org/10.1099/ijsem.0.001502.

Koch HG. 1984. Survival of the lone star tick, *Amblyomma americanum* (Acari: *Ixodidae*), in contrasting habitats and different years in southeastern Oklahoma, USA. Journal of Medical Entomology 21(1):69-79. https://doi.org/10.1093/jmedent/21.1.69.

Kordick SK, Breitschwerdt E, Hegarty B et al. 1999. Coinfection with multiple tick-borne pathogens in a Walker Hound kennel in North Carolina. Journal of Clinical Microbiology 37(8):2631-2638. https://doi.org/10.1128/JCM.37.8.2631-2638.1999.

Ma D, Lun X, Li C et al. 2021. Predicting the potential global distribution of *Amblyomma americanum* (Acari: *Ixodidae*) under near current and future climatic conditions, using the maximum entropy model. Biology 10(10):1057. https://doi.org/10.3390/biology10101057.

McClung KL, Sundstrom KD, Lineberry MW et al. 2023. Seasonality of *Amblyomma americanum* (Acari: *Ixodidae*) activity and prevalence of infection with tick-borne disease agents in north central Oklahoma. Vector-Borne and Zoonotic Diseases 23(11):561-567. https://doi.org/10.1089/vbz.2023.0009.

Mixson TR, Campbell SR, Gill JS et al. 2006. Prevalence of *Ehrlichia, Borrelia,* and *Rickettsial* agents in *Amblyomma americanum* (Acari: *Ixodidae*) collected from nine states. Journal of Medical Entomology 43(6):1261-1268. https://doi.org/10.1603/0022-2585(2006)43[1261:poebar]2.0.co;2.

Ng'eno E, Alkishe A, Romero-Alvarez D et al. 2024. Phenology of five tick species in the central Great Plains. Plos one 19(5):e0302689. https://doi.org/10.1371/journal.pone.0302689.

Noden BH, Dubie TR, Henriquez BE et al. 2022. Seasonality of ticks and prevalence of *Rickettsiae* species in *Dermacentor variabilis* and *Amblyomma maculatum* across Oklahoma pastures. Journal of Medical Entomology 59(3):1033-1041. https://doi.org/10.1093/jme/tjac028.

Padgett K, Casher L, Stephens S et al. 2009. Effect of prescribed fire for tick control in California chaparral. Journal of Medical Entomology 46(5):1138-1145. https://doi.org/10.1603/033.046.0522.

Perumalsamy N, Sharma R, Subramanian M et al. 2024. Hard ticks as vectors: the emerging threat of tick-borne diseases in India. Pathogens 13(7):556. https://doi.org/10.3390/pathogens13070556.

Peterson JR. 2012. Distribution, seasonal occurrence, abundance, and hosts of five Kansas ticks (Acari: *Ixodidae*).

Polito VJ, Baum KA, Payton ME et al. 2013. Tick abundance and levels of infestation on cattle in response to patch burning. Rangeland ecology & management 66(5):545-552. https://doi.org/10.2111/REM-D-12-00172.1.

Sirigireddy KR, Mock DC, Ganta RR. 2006. Multiplex detection of *Ehrlichia* and *Anaplasma* pathogens in vertebrate and tick hosts by real-time RT-PCR. Annals of the New York Academy of Sciences 1078(1):552-556. https://doi.org/10.1196/annals.1374.108.

Small M, Brennan RE. 2021. Detection of *Rickettsia amblyommatis* and *Ehrlichia chaffeensis* in *Amblyomma americanum* inhabiting two urban parks in Oklahoma. Vector-Borne and Zoonotic Diseases 21(5):385-387. https://doi.org/10.1089/vbz.2020.2755.

Sonenshine DE, Levy GF. 1971. The ecology of the lone star tick, *Amblyomma americanum* (L.), in two contrasting habitats in Virginia (Acarina: *Ixodidae*). Journal of Medical Entomology 8(6):623-635. https://doi.org/10.1093/jmedent/8.6.623.

Willis D, Carter R, Murdock C et al. 2012. Relationship between habitat type, fire frequency, and *Amblyomma americanum* populations in east-central Alabama. Journal of Vector Ecology 37(2):373-381. https://doi.org/10.1111/j.1948-7134.2012.00241.x.