EQUINE INFESTATION WITH COMMON NORTH AMERICAN TICKS: DIVERSITY, SEASONALITY, AND ATTACHMENT SITE PREFERENCES

By

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Abstract:

Ticks are common on horses, and the species and stages infesting horses have been documented in many regions of the world. However, recent publications characterizing equine tick infestations in North America are lacking, especially documentation of attachment site preferences and seasonality from the same geographic location throughout the year. To further understand attachment site preferences of common ticks of horses, and to document the seasonality of equine tick infestation in northeastern Oklahoma, horses from eight farms were evaluated twice a month over a one-year period. Each horse was systematically inspected beginning at the head and moving caudally to the tail. Attachment site of ticks was recorded and all ticks collected and identified to species and stage. A total of 2,731 ticks were collected; 84.1% (74/88) of the horses were infested (median=2 ticks) at one or more examinations. Five tick species were identified, including *Amblyomma americanum* (78.2%; 2,136/2,731), *Ixodes scapularis* (18.2%; 497/2,731), *Dermacentor albipictus* brown variant (2.6%; 71/2,731), *D. variabilis* (0.7%; 20/2,731), and *A. maculatum* (0.3%; 7/2,731). Most ticks were adults (83.6%; 2,282/2,731), but immature *A. americanum* (436/2,136; 20.4%), *D. albipictus* (12/71; 16.9%), and *A. maculatum* (n=1) were occasionally recovered. *Amblyomma americanum* were most often attached to the inguinal area, and *I. scapularis* and *D. albipictus* were most commonly found on the chest and axillary region ($P < 0.0001$). Ticks were found on horses in every month of the year. The largest number of ticks (638/2,731; 23.4%) were collected in May ($P < 0.0001$). *Amblyomma americanum*, primarily immature, was the only tick recovered in September, *I. scapularis* and *D. albipictus* predominated October through February, and both *A. americanum* and *I. scapularis* were common in March. In the warmer months, April through August, *A. americanum* was the most common tick, followed by *D. variabilis* and *A. maculatum*. This research confirms that ticks common on horses in North America have attachment site preferences, and that ticks infest horses in Oklahoma throughout the year, including during the winter. Additional research is warranted to fully understand the risk these infestations pose to equine health and to investigate an effective tick control for equids.

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CHAPTER I

INTRODUCTION

Ticks are important ectoparasites infesting equids worldwide and serve as vectors for many infectious diseases (Table 1, Tirosh-Levy et al., 2013). Preferred tick attachment sites, seasonality of activity, and diversity of tick species have been well characterized for other host species in North America, including dogs, cats, and white-tailed deer (Schmidtmann et al., 1998; Saleh et al. 2019) but relatively little attention has been paid to ticks on equids in this region. For example, a short duration study performed over a period of less than three months showed that *Amblyomma americanum, A. maculatum,* and *Dermacentor variabilis* are the most common ticks found on horses in the south-central United States during the summer months, but attachment sites were not reported (Duell et al., 2013). In a survey in Maryland, horses were only examined in fall and attachment site of *Ixodes scapularis* differed from that of deer, preferring the chest and axilla of the legs (Schmidtmann et al., 1998). A passive surveillance study on ticks on horses in Saskatchewan, Canada showed that the only species submitted were *D. variabilis* from April through August, *D. andersoni* from April through June, and *D. albipictus* from February through May (Schvartz et al., 2015). *Haemaphysalis longicornis* (Asian longhorned tick), a newly established tick found as far west as Arkansas, has also recently been reported on horses in the United States (Beard et al.,

2018). With the introduction of this new exotic tick it is important to fully understand the risk that tick infestations pose to horses.

One of the most important risks tick pose is transmission of tick-borne infections resulting in illness. Pathogens vectored by common North American ticks and associated with equine disease include *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Ehrlichia* spp., and *Rickettsia* spp. (Table 2, Madigan et al., 1990; Stiller et al., 2018; Chang et al., 2000; Duncan et al., 2020). Novel and recognized equine piroplasmosis agents, including *Theileria equi*, have also been reported in the United States and associated with the presence of less common ticks including *A. cajennense* and *D. nitens* (Wise et al., 2014; Knowles et al., 2018). Research reported in this thesis was undertaken to determine diversity, seasonality, and attachment site preferences of common North American ticks on horses throughout the entire year.

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CHAPTER II

REVIEW OF LITERATURE

Overview

Equine tick infestations are common worldwide, with ticks identified on 25% to nearly 100% of horses examined depending on the time of year, habitat, number of examinations, and management of the animals included in each study (Table 1). Ticks are important as both blood-sucking parasites and vectors of pathogens, including agents of equine piroplasmosis, a transboundary disease with serious equine health and trade implications (Scoles et al., 2011; Wise et al., 2014). Surveys of horses for ticks have been conducted throughout the world, but the species of ticks, prevalence of infestation, attachment site preferences, and seasonality of risk is understudied in the United States (Schmidtmann et al., 1998; Schvartz et al., 2015; Duell et al., 2013). Common ticks infesting horses in the United States include: *Amblyomma americanum, Amblyomma maculatum, Dermacentor albipictus, Dermacentor variabilis, Ixodes scapularis, Ixodes pacificus*, and *Otobius megnini*; other, less common species are also occasionally identified (Bishopp and Trembley, 1945; Barnard et al., 1989; Edwards et al., 2011; Duell et al., 2013; Carmichael et al., 2014; Maestas et al., 2020).

Tick attachment site preferences are often recognized for different hosts, and limited surveys from equids show that there are some differences between equine tick

attachment site preferences and those reported from dogs, cats, and white-tailed deer (Schmidtmann et al., 1998; Duell et al., 2013; Saleh et al., 2019). Characterizing attachment sites for common ticks on horses allows horse owners and veterinarians to focus efforts during wellness examination and to remove these parasites and help prevent disease transmission, respectively. Current data suggests that the most common sites of tick attachment on horses are the head, ears, axillary, and inguinal regions (Schmidtmann et al., 1998; Zeringota et al., 2013; Njaa, 2017).

Equine ticks in North America

Amblyomma americanum

Amblyomma americanum, the lone star tick, is a three-host tick found most often in wooded areas with a dense understory and adequate leaf litter to protect developing stages from desiccation (Paddock et al., 2003). Adult lone star ticks aggressively seek hosts and infest a large range of medium and large sized mammals, including white-tailed deer. Immature stages also infest deer and other medium and large mammals as well as ground nesting birds (Bishopp and Trembley, 1945). Until the 1990s, populations of *A. americanum* were primarily found in high number in the southcentral and southeastern United States, but the range has since expanded and this tick is now found in most eastern and midwestern states (Bishopp and Trembley, 1945; Saleh et al., 2021). Adult lone star ticks are active earlier in the year (March–August), while nymphs have a broader activity range (March–October), and larvae are active in the later summer and early fall (July–October) (Koch et al., 1982; Jackson et al., 1996).

The primary known equine health issues associated with *A. americanum* are painful, localized reactions from the attachment site and, in sensitized horses, allergic disease (Tritschler, 1965). *Amblyomma americanum* is the main vector for both *Ehrlichia ewingii* and *E. chaffeensis* (Saleh et al., 2021), although neither agent has been shown to cause disease in horses to date. Antibodies to *Ehrlichia* spp. are common in horses from Oklahoma and are considered most likely due to infection with a novel, not-yet-identified *Ehrlichia* sp. (Carmichael et al., 2014). *Amblyomma americanum* also transmit spotted fever group *Rickettsia* spp. (SFGR), including *R. amblyommatis*, which is considered largely non-pathogenic, and *R. rickettsii,* causative agent of Rocky Mountain spotted fever (Karpathy et al., 2016; Levin et al., 2017). Equine disease due to SFGR is only rarely described in the United States (Freese and Sheats, 2019), and recent attempts to induce disease with experimental inoculation of horses have not been successful (Ueno et al., 2016).

Amblyomma maculatum

Amblyomma maculatum, the Gulf Coast tick, is a large three-host tick that thrives in open areas, including open savannah and scrubland adjacent to wetlands, coastal wetlands, drier grasslands, and tall-grass prairies (Paddock et al., 2015; Saleh et al., 2021). Adult Gulf Coast ticks feed on a variety of hosts including livestock, companion animals, mammalian and avian wildlife, and humans, while immature stages prefer birds and small mammals (Maestas et al., 2020; Saleh et al., 2021). *Amblyomma maculatum* historically prefer high humidity habitats of the Gulf Coast of the United States but over the last century have expanded to Oklahoma, Kansas, and Arkansas, where they have

readily adapted to drier environments, as well as up the Atlantic Coast as far north as Connecticut (Paddock et al., 2015; Saleh et al., 2021; Molaei et al., 2021). Activity of *A. maculatum* occurs in three peaks, with adults appearing first, then nymphs, followed by larvae (Teel et al., 2010; Saleh et al., 2021). Host seeking adult *A. maculatum* can be found during the heat of the day, in open areas from March through September. Seasonal activity of immature *A. maculatum* is less clear and in need of further investigation.

Amblyomma maculatum have been responsible for economic loss in the livestock industry for many years due to dermal trauma at the attachment site which may lead to inflammation, secondary infections, and abscess formation, and can predispose cutaneous myiasis (Teel et al., 2010). The preference of the adults to attach to the inner and outer surface of the ear pinnae and resulting inflammation leads to the devastating condition "gotch ear" (Edwards, 2011; Paddock et al., 2015). Equine pathogens transmitted by the Gulf Coast tick have not been described, but this tick does serve as a key vector for *Hepatozoon americanum*, causative agent of American canine hepatozoonosis, and *Rickettsia parkeri*, a SFGR that causes disease in people. In addition, some data suggest *A. maculatum* may be able to transmit *E. chaffeensis, E. ruminantium,* and Panola Mountain *Ehrlichia* sp. (Paddock et al., 2015), but the role these pathogens play in equine health is not known.

Other *Amblyomma* spp. of importance to horses in the United States

Although rarely reported from horses in the United States, *A. cajennense*, the cayenne tick, is a three-host tick that readily feeds on horses. Cayenne ticks are found in the far southern United States, Mexico, Central America, and South America and were

shown to experimentally transmit *Theileria equi* following an outbreak in southern Texas (Scoles et al., 2011). The tropical bont tick, *A. variegatum*, is a three-host tick originally from Africa that has been established in several Caribbean countries for many years. While established populations have not been reported from the continental United States, introduction of tropical bont ticks is considered a threat to mainland North America and cattle egrets are thought to be a major transport host. This tick species is important for many diseases, including heartwater (*Ehrlichia ruminantium*), which affects ruminants, and dermatophilosis (*Dermatophilus congolensis*), a secondary infection that develops following skin damage by tick feeding and localized immunosuppression (Camus et al., 1990).

Dermacentor albipictus

Dermacentor albipictus, known commonly as the winter tick, is a one-host tick that can survive and reproduce in a variety of environments such as forest openings and a closed canopy, deciduous forest depending on weather and microsite conditions (Addison et al.,1988; Addison et al., 2016). Winter ticks feed primarily on large mammals such as moose, white-tailed deer, other wild ruminants, cattle, and horses (Teel et al., 1990; Samuel, 2004); occasionally infestations are reported from companion animals or people (Duncan et al., 2020). *Dermacentor albipictus* are widely distributed in North America, from Canada to Mexico (Bishopp and Trembly, 1945). In the northern part of their range, *D. albipictus* is strongly associated with moose and elk populations and most commonly occurs as the ornate phenotype. In more southern areas, the brown variant predominates and white-tailed deer, cattle, and horses are more important hosts (Patrick and Hair, 1975;

Calvente et al., 2020). Larvae seek out a host from September through November, often attaching together in large numbers (Lindquist et al., 2016). Once on the host, they feed and molt to nymphs, then again to adults, completing their life cycle on the same host. Engorged females will detach to lay eggs in late winter or early spring of the following year (Lindquist et al., 2016).

The primary health importance of *D. albipictus* is the heavy infestations that lead to weight loss, alopecia, anemia, and death (Calvente et al., 2020). In addition, *D. albipictus* has been implicated as a vector of *Anaplasma marginale*, causative agent of anaplasmosis in cattle, and *Babesia duncani*, an emerging human babesiosis agent, and has been confirmed experimentally as a vector of *Babesia caballi* in equines (Stiller et al., 1980; Ewing et al., 1997; Swei et al., 2019).

Dermacentor variabilis

Dermacentor variabilis, the American dog tick, is a three-host tick commonly found questing on short grass, along forest boundaries, and along the edges of trails and roadways (Saleh et al., 2021). One of the most common tick species found on cats and dogs in the United States, this tick is also common on cattle and horses and has been documented to be the second most common tick found on horses in the summer in Oklahoma (Duncan et al., 2021; Duell et al., 2013). Immature stages of *D. variabilis* feed on rodents and other small mammals, and adults are found on a variety of medium to large mammals, including humans (James et al., 2006). *Dermacentor variabilis* has a wide geographic distribution in North America, spanning most of the eastern United States from Florida and the Gulf of Mexico up to southern Canada; an isolated western

population is also found along the Pacific Coast (Saleh, et al., 2021). Historically *D. variabilis* was considered largely absent from the Rocky Mountain region but reports back to the 1930s indicate the range of *D. variabilis* overlaps with *D. andersoni*, and recent surveys confirm that *D. variabilis* is common in this region (Gibbons, 1939; Duncan et al., 2021). Adults of *D. variabilis* usually appear as early as April and peak in May, June, and July, with immature stages following a month or two later and activity of all stages persisting through the early fall (James et al., 2006; Duncan et al., 2021). All stages become inactive by late summer to early fall and then overwinter to emerge the following spring (Cooley, 1932). In the southern part of the *D. variabilis* range, the life cycle may be completed in one year, and in more northern areas, two years is often required (Nicholson et al., 2019).

Dermacentor variabilis has been associated with tick paralysis in horses (Carroll et al., 1986). In addition, this tick is an important vector of SFGR including *R. montanensis*, *R. bellii*, *R. amblyommatis*, *R. rhipicephali*, and less commonly *R. rickettsii* (Hecht et al. 2019). Although *R. rickettsii* has been reported to be found in less than 0.1% of *D. variabilis*, and in some surveys is not detected at all, it is still considered one of the most important disease agents vectored by this tick due to its high pathogenicity (Duncan et al., 2021; Saleh et al., 2021). *Ehrlichia chaffeensis* and *E. ewingii* have been documented occasionally in wild-caught *D. variabilis* but the role that these pathogens play in equine health, if any, is unclear (Steiert et al., 2002; Fritzen et al., 2011; Wright et al., 2014). *Dermacentor variabilis* can also transmit *T. equi* and *B. caballi*, the agents responsible for equine piroplasmosis (Stiller et al., 2002). *Theileria equi* can also be

transferred intrauterine to the fetus throughout the life of the mare creating a serious economic impact to horse breeders (DeWaal, 1992).

Other *Dermacentor* spp. of importance

Dermacentor andersoni, the Rocky Mountain wood tick, is mainly restricted to the Rocky Mountain region of the United States. This tick is responsible for transmission of Colorado Tick Fever virus, *R. rickettsii*, *A. marginale*, and *F. tularensi*s, none of which are commonly associated with equine disease (Marchiondo et al., 2019). *Dermacentor occidentalis*, the Pacific Coast tick, is the most widely distributed tick in California and is the primary vector of the agent of Pacific Coast tick fever, an unclassified SFGR that causes disease in people but is not known to infect horses (Paddock et al., 2018). *Dermacentor nitens*, the tropical horse tick, is limited in the United States to the southern regions of Texas and Florida. Like *D. albipictus, D. nitens* is a one-host tick and in areas where this tick is prevalence, high intensity infestations with several thousand ticks are common (Rodrigues et al., 2017). *Dermacentor nitens* can vector *B. caballi*, an agent causing equine piroplasmosis (Schwint et al., 2008). Another species, *D. reticulatus,* is important in Europe but has not been reported in North America; it infests over 60 known hosts, is able to survive unfavorable conditions, has a high reproduction rate, and is a vector for important pathogens for both small and large animals including: *B. canis, B. caballi*, *T. equi, A. marginale, Rickettsia* spp., *F. tularensis,* and *Coxiella burnettii* (Folfvari et al., 2016).

Ixodes scapularis

Ixodes scapularis, commonly called the black-legged tick or deer tick, is a three-host tick; all stages prefer to be in dense leaf litter where they can be protected from the environmental factors detrimental to their survival (Lindsay et al., 1999). Adult *I. scapularis* rely on white-tailed deer to maintain their population but will readily feed on other medium and large-sized mammals (Oliver et al., 1993; Wilson, 1998;). Nymphal and larval stages have a diverse breadth of hosts and may exhibit different host preferences depending on geographic location, especially in the nymphal stage (Mather et al., 1989; Oliver et al., 1993; Saleh et al., 2021). In the northern United States and in Canada, immature *I. scapularis* commonly feed on small mammals, including rodents and shrews, and in the southern United States lizards are a key supportive host. Adults become most active between 5–15 ˚C, translating to northern populations peaking in October and November with a second peak occurring in March and April, while in states such as South Carolina and Florida adults peak January through March (Ogden et al., 2004; Saleh et al., 2021). Nymphs will be active once the temperature reaches 15–25˚C and shortly after larvae will emerge (Ogden et al., 2004). *Ixodes scapularis* is found throughout the eastern half of the United States, with populations extending from Florida across to central Texas, north to eastern North Dakota, and into southern Canada (Saleh et al., 2021). Another related species, *I. pacificus* occurs along the Pacific Coast of the United States and into British Columbia, Canada (Eisen et al., 2016).

Ixodes scapularis is an important tick for both animal and human health. They vector important pathogens including *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, and *Ehrlichia muris eauclairensis* (Saleh et al., 2021). Seroprevalence of *B. burgdorferi*

in dogs, horses, and humans is increasing, most likely due to the increasing range of *I. scapularis* (Divers et al., 2018). This range expansion is important for horses since there is no approved vaccine for Lyme disease and the morbidity after *B. borrelia* infections is unknown in horses (Divers et al., 2018). Although death from *A. phagocytophilum* is rare in horses, it is still a pathogen of concern and tick control is a large part of controlling the disease (Uehlinger et al., 2011). *Anaplasma phagocytophlium* and *B. burgdorferi* are considered the most common tick-borne diseases that affect horses in the United States (Magnarelli et al., 2000).

Otobius megnini

Otobius megnini, commonly known as the spinose ear tick, is a soft tick which only parasitizes one host in its life cycle (Saleh et al., 2021). Only immature stages are parasitic and, as the common name suggests, all stages feed within the external ear canal of the host which includes horses, cattle, goats, sheep, wild ungulates, and sometimes cats and dogs (Keirans et al., 2003; Saleh et al., 2019). *Otobius megnini* prefers hot and dry environments and can be found primarily in the southwestern United States and Mexico (Saleh et al., 2021). Although infestations occur more often in the winter and spring, ticks may be found on hosts year-round (Saleh et al., 2021).

Otobius megnini is not considered an important vector of any known pathogens in North America (Ewing et al., 1990). Although there have been reports of detection of *Coxiella burnetii*, *Francisella tularensis*, and *R. rickettsii* in *O. megnini*, the spinose ear tick is not considered a competent vector for these agents (Bowman, 2013). In horses, *O. megnini* is the causative agent of equine otoacariasis, an at-times serious condition that

can cause muscle fasciculations, neuromuscular dysfunction, injury, and death (Madigan et al., 1995; Rajakaruna et al., 2019; Miller, 2020).

Introduced Ticks

Two exotic ticks of importance to equine health that have been recently introduced or re-introduced into the United States are *Haemaphysalis longicornis* and *Rhipicephalus microplus*. *Haemaphysalis longicornis,* the Asian longhorned tick, is native to East Asia and was reported in the United States for the first time in 2017 (Rainey et al., 2018; USDA, 2020). Since the initial report, *H. longicornis* has been identified in 15 different states, from collections dating back to 2010, and on a variety of hosts including humans, dogs, cats, cattle, horses, sheep, white-tailed deer, coyotes, raccoons, and opossums (Duncan et al., 2020; Saleh et al., 2021). *Haemaphysalis longicornis* is a competent vector for several pathogens in Asia, but to date only *Theileria orientalis*, a pathogen of cattle, has been confirmed to be naturally transmitted by this tick in the United States (Beard et al., 2018; Dinkel et al., 2021). Nevertheless, the longhorned tick is a species of concern for horses and other animals and needs to continue to be monitored.

Rhipicephalus microplus, the southern cattle tick, was eradicated from the United States in 1943 and reintroduction has been estimated to potentially cost the cattle industry millions of dollars (Anderson et al., 2010). In recent years, wild caught larvae of *R. microplus* have been collected in the Coastal Wildlife Corridor in southern Texas, indicating completion of the life cycle continues in this area (Osbrink et al., 2020). Eradication efforts are still being made in order to prevent an outbreak and keep cattle

free of bovine babesiosis (USDA 2018). *Rhipicephalus microplus* readily feeds on horses, and infested horses could potentially transport this tick from southern Texas to other areas of the country. In addition, southern cattle ticks serve as a vector of the agents responsible for equine piroplasmosis, a disease that affects horses in many parts of the world (Stiller et al., 2002).

Attachment site preferences of ticks in North America

Limited data are available regarding attachment site preferences for *Amblyomma* spp., *Dermacentor* spp., and *Ixodes scapularis* on different hosts in North America. Attachment site predispositions for *Amblyomma* spp. have been described in dogs, cats, cattle, deer, and, minimally, in horses. In nursing beef calves, the preferred attachment sites for male, female, and nymphal *A. americanum* is the axillary, inguinal, and perianal areas (Barnard et al., 1989). In dogs, the preferred attachment site is the abdomen, axillary, and inguinal area, and in cats *A. americanum* prefers the tail and perianal region (Saleh at al., 2019). In white-tailed deer, the preference for all stages of *A. americanum* is the head, ears, and neck (Carroll et al., 2002). To the authors' knowledge, no reports describe attachment site preferences for *A. americanum* in horses in the United States, although *A. maculatum* favors the ears and head of all the hosts they infest (Njaa, 2017).

Attachment site data has shown that *D. variabilis* prefers the dorsal aspect of cats and dogs, and particularly the back (Little et al., 2018; Saleh et al., 2019). In white-tailed deer, *D. albipictus* has been reported almost solely from the head although on moose and elk, the majority of the body may be infested (Samuel, 2004; Baer-Lehman et al., 2012). Literature suggests that like *D. variabilis*, *D. andersoni* and *D. occidentalis* will attach to

the dorsal aspect of cattle (Wilkinson, 1972; Cadet and Bolla, 2007). In horses, *D. nitens* preferentially attaches to the ears and nasal diverticulum (Zeringota et al., 2013).

Most attachment site data for North American *Ixodes* has been collected for *I. scapularis.* In white-tailed deer and cattle, the primary sites of *I. scapularis* attachment are the head, ears, neck, and brisket (Barnard, 1981; Carroll et al., 2002). Research shows that *I. scapularis* attachment sites are similar in cats and dogs, where they prefer the head, ears, and neck (Little et al., 2018; Saleh et al., 2019). Unlike with other hosts, an equine-focused study in Maryland showed that adult *I. scapularis* were largely absent from the ears and neck of horses, and that 84% of females were attached to the chest, axillae, inguinal, or under the mandible (Schmidtmann et al., 1998).

Conclusions and research objectives

Ticks infesting horses in North America are understudied. To gain a better understanding of ticks and associated diseases important to equine health, we need to document the diversity, seasonality, and attachment site preferences of tick species infesting horses throughout the United States. There is a growing foundation of knowledge about these key issues in other hosts in North America, and, for equine ticks, other countries. For the present study, we addressed the risks ticks pose to horses year-round by completing the following research objectives:

RESEARCH OBJECTIVES

1. Determine diversity and seasonality of tick species infesting horses year-round in Oklahoma
2. Characterize the attachment site preferences of tick species found on horses in this

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CHAPTER III

EQUINE ATTACHMENT SITE PREFERNCES OF COMMON NORTH AMERICAN TICKS: *AMBLYOMMA AMERICANUM, DERMACENTOR ALBIPICTUS, AND IXODES SCAPULARIS*

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Abstract

Background: Ticks are common on horses, but recent publications characterizing equine tick infestations in North America are lacking. **Methods**: To further understand attachment site preferences of common ticks of horses, and to document the seasonality of equine tick infestation in northeastern Oklahoma, horses from eight farms were evaluated twice a month over a one-year period. Each horse was systematically inspected beginning at the head and moving caudally to the tail. Attachment site of ticks was recorded and all ticks collected and identified to species and stage. **Results**: Horses (26 male and 62 female) enrolled in the study ranged in age from 1 to 23 years (mean=12, 95% CI 11–13). A total of 2,731 ticks were collected; 84.1% (74/88) of the horses were infested (median=3 ticks) at one or more examinations. Five tick species were identified, including *Amblyomma americanum* (78.2%; 2,136/2,731), *Ixodes scapularis* (18.2%; 497/2,731), *Dermacentor albipictus* brown variant (2.6%; 71/2,731), *Dermacentor variabilis* (0.7%; 20/2,731), and *Amblyomma maculatum* (0.3%; 7/2,731). Most ticks were adults (83.6%; 2,282/2,731), but immature *A. americanum* (436/2,136; 20.4%), *D. albipictus* (12/71; 16.9%), and *A. maculatum* (n=1) were occasionally recovered. *Amblyomma americanum* were most often attached to the inguinal area, and *I. scapularis* and *D. albipictus* were most commonly found on the chest and axillary region ($P < 0.0001$). Ticks were found on horses in every month of the year. The largest number of ticks (638/2,731; 23.4%) were collected in May ($P < 0.0001$). *Amblyomma americanum*, primarily immature, was the only tick recovered in September, *I. scapularis* and *D. albipictus* predominated October through February, and both *A. americanum* and *I. scapularis* were common in March. In the warmer months, April through August, *A.*

americanum was the most common tick, followed by *D. variabilis* and *A. maculatum*.

Conclusions: This research confirms that ticks common on horses in North America have attachment site preferences, and that ticks infest horses in Oklahoma throughout the year, including during the winter. Additional research is warranted to fully understand the risk these infestations pose to equine health.

Key Words: *Amblyomma*, attachment site, *Dermacentor*, equine, *Ixodes*, tick

Background

Ticks commonly infest horses in North America, causing localized inflammation, dermal trauma, and sometimes resulting in transmission of pathogens or systemic reactions [1–5]. Ixodid ticks most frequently identified from horses in the USA include *Ixodes scapularis*, which transmits *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, both of which have been shown to cause equine disease; *Amblyomma americanum*, which can induce localized reactions; and *Dermacentor variabilis*, a species associated with equine tick paralysis and experimentally competent for transmission of *Theileria equi* [1, 3, 6–10]. Although considered of greater concern for cattle, horses in some areas of the USA can serve as hosts to *A. maculatum*, resulting in equine gotch ear, as well as the newly established *Haemaphysalis longicornis* and occasionally re-introduced *Rhipicephalus* (*Boophilus*) spp. ticks [6, 11, 12]. Novel and recognized equine piroplasmosis agents have also been reported in the region [13, 14]. Despite the importance of ticks to equine health, recent comprehensive tick surveys of North American horses are sparse.

Different species of ticks display attachment site preferences on different hosts. For example, on dogs and cats, *I. scapularis* most commonly attaches to the head, ears,

and neck, whereas *A. americanum* is more often found ventrally [15]. On white-tailed deer, over 85% of *I. scapularis* are attached to the ears, head, neck, and cranial thorax, while *A. americanum* are primarily found on the head and ears as well as ventrally on the abdomen, inguinal, and perianal region; 75% of adult *A. americanum* are found attached to the left side of deer [1, 16]. On horses, most (84%) *I. scapularis* females are found attached to the chest, the axillary and inguinal regions, and below the chin, and adult *D. variabilis* prefer the tail and mane [1, 7]. In Israel, *Hyalomma* spp. and *Rhipicephalus turanicus* are most commonly attached to the inguinal region of horses, while *Haemaphysalis parva* is most often found on the neck and chest [17]. Equine attachment site preferences have not been reported for *A. americanum* or *D. albipictus*.

The seasonal activity of species and stages of ticks varies, leading to fluctuations in equine infestation risk throughout the year. An earlier survey conducted from May through July of 2010 on Oklahoma horses described finding adult *A. americanum* earlier in the summer and adults of both *D. variabilis* and *A. maculatum* in later summer months [3]. *Ixodes scapularis* was collected from horses examined in Maryland from mid-October through November [1]. Tick collections from feral horses at Assateague Island National Seashore in April of 1987 and 1988 revealed mixed infestations with *A. americanum*, *D. variabilis*, and *I. scapularis* although intensity of infestation was not reported and only the head and neck were examined [18]. Both *A. americanum* and *D. variabilis* were identified on horses in Kentucky examined May through August of 2008 [19]. Some ticks known to infest horses in North America, such as *I. scapularis* and *D. albipictus*, are active in the fall and winter when few surveys are reported [1, 20]. To determine attachment site preferences of common equine ticks in North America, and to

confirm the diversity and seasonal activity of ticks infesting horses in central Oklahoma, we examined horses on 8 premises twice a month over the course of 12 months and collected and identified their ticks.

Materials and Methods

Premises with horses were selected for participation based on the presence of at least three resident horses on site; expected exposure to habitat with ticks was not considered. Horses were only enrolled if they resided on the premise, did not travel out of their county of residence, and were > 1 year of age at time of enrollment; however, herds were open to both travel and introduction of new horses. All protocols were approved by the Institutional Animal Care and Use Committee at Oklahoma State University and permission to examine horses obtained through approved owner consent forms. Acaricide use was not specifically restricted or encouraged during the study, and owner acaricide practices were not recorded. Horses were examined for a full calendar year (12 months) from September 2019 through March 2021; horses continued to be enrolled as the study progressed, and tick exams were performed twice a month at each location for a full calendar year. Horses were systematically examined by parting the hair and closely inspecting the skin beginning at the head and moving caudally to the tail with an examiner positioned on either side of the horse. Once the dorsum and perianal regions were thoroughly scanned the exam continued distally along the chest and forelegs, caudally along the axillary, ventral, and inguinal area, and ended moving along the distal hind legs [3]. Since horses were not sedated, exams did not include looking in ear canals. All stages of ticks were collected; when ticks were found, attachment site was recorded on a biopsy chart, ticks were removed, placed in snap cap vials, and labeled by horse and

attachment site. Ticks were stored in 70% ethanol and identified to species and stage using standard morphological keys [20–26]. When necessary, morphologic identification was confirmed by *16S* rRNA gene sequence as previously described [15].

For data analysis, attachment sites were divided into 7 body regions, namely: head (1); neck (2); chest, axillary region, and cranial abdomen (3A); caudal abdomen and inguinal region (3B); legs (4); dorsal back (5); and tail and perianal region (6). Descriptive statistics (mean, range, proportion, and 95% confidence intervals (CI)) were calculated with Microsoft Excel (Microsoft Office Professional Plus 2016). Fisher's exact test or chi-square tests with a significance of alpha = 0.05 was used to compare age class and sex of infested horses, seasonality of infestation, and tick attachment site preferences, including ventral and dorsal, left and right, and body region. The mean number of ticks collected from horses at each of the 8 properties were compared by one-way ANOVA followed by Tukey HSD.

Results

Tick collection from horses.

A total of 88 horses (26 male, 62 female) from 8 different ranches in 3 counties in Oklahoma (Payne, Pawnee, Logan) were enrolled in the study, with ages ranging from 1-23 years (mean 12, 95% CI 11–13). Several horses were sold or relocated in the course of the one-year study; 54 horses were examined for a full 12 months, and 34 horses were examined 1–20 times for a total of 1,661 equine tick examinations. A total of 2,731 ticks were collected. Over the entirety of the study, 84.1% of the horses (74/88; 95% CI 74.8–91.0%) were infested with ticks at one or more examinations (median=3 ticks), consisting of 45.2% (1,233/2,731; 95% CI 43.3–47%) female ticks; 38.4% (1,049/2,731; 95% CI

37–40%) male ticks; 10.2% (279/2,731; 95% CI 9.1–11.4%) nymphs; and 6.2% (170/2,731; 95% CI 5.3–7.1%) larvae. Sex and age class of horses did not significantly influence tick infestation (P=0.543 and 0.563, respectively). The mean numbers of ticks were significantly different at the different properties (F=35.07, df=7, P<0.0001; Table 3).

The tick species identified were *Amblyomma americanum* (78.2%; 2,136/2,731; 95% CI 76.7–79.8%); *Ixodes scapularis* (18.2%; 497/2,731; 95% CI 16.8–19.7%); *Dermacentor albipictus* (2.6%; 70/2,731; 95% CI 2–3.2%); *Dermacentor variabilis* (0.7%; 20/2,731; 95% CI 0.5–1.1%); and *Amblyomma maculatum* (0.3%; 7/2,731; 95% CI 0.1–0.5%). A majority of ticks collected (83.6%; 2,282/2,731; 95% CI 82.2–85%) were adults, but nymphs of *A. americanum* (12.5%; 266/2,136; 95% CI 11–13.9%), *D. albipictus* (16.9%; 12/71; 95% CI 9.1–27.7%), and *A. maculatum* (14.3%; 1/7; 95% CI 0.36–57.9%) were also identified. *Amblyomma americanum* (8%; 170/2,136; 95% CI 6.9–9.2%) were the only larval ticks collected (Table 4).

Attachment site preferences.

Attachment site differed between tick species collected. *Amblyomma americanum* adults were most often found in the inguinal area while nymphs and larvae were most commonly seen on the neck, chest and legs (P<0.0001). *Ixodes scapularis* adults, the only stage of this species recovered from horses, and adults of *D. albipictus*, were most commonly found on the chest and axillary region (P<0.0001); *D. albipictus* nymphs were also most common in this region (P = 0.0034) (Figure 2). *Dermacentor albipictus* was more commonly identified on the left side (P<0.0001); although ticks were more numerous on the left side, significant attachment side preferences (left versus right) were

not evident for *A. americanum* (*P*=0.069) or *I. scapularis* (*P*=0.823). All three species with adequate numbers for evaluation (*A. americanum, I. scapularis, D. albipictus*) were significantly more likely to be attached ventrally ($P < 0.0001$).

Seasonality of tick infestations.

In fall, winter, and early spring (October 2019–March 2020; October 2020–March 2021), 633 ticks were collected, with 62.5% (55/88; 95% CI 51.5–72.6%) of the study population infested (median=2 ticks) at one or more examinations. Tick species found during these cooler months included *Ixodes scapularis* (76.8%; 486/633; 95% CI 73.3–80%), *Dermacentor albipictus* brown variant (11.1%; 70/633; 95% CI 8.7–13.8%), and *Amblyomma americanum* (12.2%; 77/633; 95% CI 9.7–15%) (Table 3, Figure 1).

In the warmer months (April–September 2020), 2,098 ticks were collected, with 72.7% (64/88; 95% CI 62.2–81.7%) of the study population infested (median=3 ticks) at one or more examinations. Tick species found during these warmer months included *A. americanum* (98.2%; 2,060/2,098; 95% CI 97.6–98.8%), *D. variabilis* (1.0%; 20/2,098; 95% CI 0.6–1.5%), and *A. maculatum* (0.3%; 7/2,098 95% CI 0.1–0.7%) (Table 3, Figure 1). A greater proportion of ticks ($P < 0.0001$; X^2=785.9; df=1) were collected from horses in the warmer months (2,098/2,731; 76.8%; 95% CI 75.2–78.4%) than in the cooler months (633/2,731; 23.2%; 95% CI 21.6–24.8%), but Fisher's test showed the prevalence of infestation did not differ between the two time periods (*P*=0.1973).

Discussion

The present research confirms that, as reported for other hosts, attachment site of ticks on horses varies by tick species. This phenomenon is well recognized for *Otobius megnini*, a soft tick commonly found in the external ear canal of horses in the western

USA, but less established for common ixodid species [27]. The finding in the present study that *I. scapularis* adults commonly attach to the chest of horses agrees with those previously reported for horses in Maryland in the eastern USA [1]. In contrast, *A. americanum* was most frequently found in the inguinal region, similar to data from dogs and cats confirming this species prefers to attach ventrally, and humans, where a majority of *A. americanum* attach below the waist [15, 28, 29]. A significant preference for the left side, as has been documented in white-tailed deer, was not evident in our *A. americanum* equine data [16]. However, the present study did document a left side bias for *D. albipictus* attachment which has not, to our knowledge, been previously reported [30]. Together, these data and other studies indicate that ticks may be found on several regions of horses, supporting the need for complete external parasite examination when attempting to accurately characterize equine tick infestations [1, 17].

Similar to earlier reports from examining horses at certain times of the year, the most common tick species to infest horses in this region of North America, as in most of the eastern United States, are *A. americanum*, *I. scapularis*, and *Dermacentor* spp. [1,3]. The predominance of *A. americanum* in the present study reflects the location of the study site in the southern USA where this tick is common [3, 31]. Similarly, the brown variant of *D. albipictus*, formerly referred to as *D. nigrolineatus*, and not the ornately patterned variety, is more commonly recovered from horses in this region although both variants have been reported [32]. *Ixodes scapularis* is also a frequent parasite of horses although only adult *I. scapularis* are routinely found on mammals in the southern USA [1, 33, 34]. Both *D. variabilis* and *A. maculatum* were relatively rare on horses in the present study,

accounting for less than 1% of all ticks recovered, a finding likely due to the limited number of equine premises visited.

The data from the present study confirms that ticks infest horses year-round, including in the winter months (Table 3). To our knowledge this is the first study confirming tick infestations on horses every month of the year in North America. As is seen in infestations in other domestic animals, the seasonality of equine infestations corresponds with established phenology for each tick species in the region, with *A. americanum* adults found in highest numbers on horses in the spring and summer, immature *A. americanum* most common in August and September, and both *D. albipictus* and *I. scapularis* predominating in the cooler fall and winter months [27, 28, 31]. Although a previous study suggested that both *D. variabilis* and *A. maculatum* are also commonly found on horses in the summer, the present study did not recover adequate numbers of either species to confirm this finding [3]. Seasonal differences in timing of peak activity between tick species leads to waxing and waning intensity of infestations, but tick populations do not entirely disappear, resulting in a year-round risk.

The findings from the present study have some limitations. Although occasionally identified in the region, *Otobius megnini* was not recovered from any horse, likely because this species is found in the external ear canal and we did not sedate horses for thorough otic examination [27]. The low numbers of *A. maculatum* and *D. variabilis* recovered was surprising but may be due to the limited number of premises with horses enrolled (n=8) or year-to-year fluctuations in tick populations related to precipitation or other factors [35]. Habitat may also have influenced the findings; both *A. americanum* and *I. scapularis* prefer wooded habitat with dense understory, whereas *D. variabilis* and

A. maculatum are more commonly found in open areas, overgrown fields or meadows [27]. In areas where Lyme disease is endemic or emerging, horses in pastures with oak trees are significantly more likely to be seropositive for *B. burgdorferi* [36]. Tick control practices were not recorded for the horses surveyed in the present study and likely varied between premises, but ticks were still commonly found (Table 3). Although the reason for the significant differences in mean number of ticks at the different properties was not identified, we suspect differences in both vegetation and management of horses to be responsible. Although attachment site preferences for the species considered are broadly applicable, additional research is warranted to fully appreciate the risk all tick species in North America pose to equine health. As with small animals, horses may benefit from year-round tick control. Unfortunately, available options for equine tick control are limited, require frequent re-application, and may have safety concerns, suggesting tick infestations will continue to be a challenge for horse owners.

Declarations

Ethics approval and consent to participate. All protocols were approved by the Institutional Animal Care and Use Committee at Oklahoma State University and permission to examine horses obtained through approved owner consent forms.

Consent for publication. Not applicable.

Availability of data and materials. The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests. The authors declare that they have no competing interests.

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Authors' contributions. KS designed the study, identified study sites, enrolled horses, collected and analyzed data, and drafted the manuscript. ML, AG, KD, and MI provided support in collecting and identifying ticks, and in collating, analyzing, and interpreting data. SL provided support in study design, data analysis, and writing the manuscript. All authors read and approved the final manuscript.

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CHAPTER IV

CONCLUSION

Currently, only limited, seasonal surveys have been reported describing ticks infesting horses in North America (Schmidtmann et al., 1998; Duell et al., 2013). To the authors knowledge the present study in this thesis is the first survey in the United States that followed the same subset of horses year-round to investigate tick diversity, seasonality of risk, and attachment site preferences. Our overall findings proved that ticks are common on horses every month of the year in this region, and that the common ticks infesting horses are *Amblyomma americanum, Dermacentor albipictus,* and *Ixodes scapularis*, with *Dermacentor variabilis* and *Amblyomma maculatum* also present.

Attachment site data for many other hosts have been well documented including dogs, cats, and white-tailed deer (Schmidtmann et al., 1998; Saleh et al. 2019), but data is lacking on attachment site of common ticks infesting horses in North America. The present study has helped set the groundwork for understanding attachment site data in equids, and the results underscored important differences in attachment site preferences on horses for common North American ticks. More research is needed to determine attachment site preferences for *A. maculatum, D. variabilis,* and *O. megnini*; the current survey cannot address this question as these species were not found in large numbers in the current survey and ears were not thoroughly examined.

Although attachment site preferences and seasonality for the species considered are broadly applicable between host species, additional research is warranted to fully appreciate the risk all tick species in North America pose to equine health. Horses may benefit from year-round tick control and would benefit from a broader array of tick control options. Currently, available options for equine tick control are limited in scope, require frequent re-application, and may have safety concerns, suggesting that controlling tick infestation on horses will continue to be a struggle. Continuing tick surveys on equids can also support surveillance for newly introduced tick species to North America.

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APPENDIX

Table 1. Representative published reports of ticks recovered from horses

Study population (N; %)	**Ticks identified (n; tick stages[a])**	**Geographic location**	**Reference**
Ranch horses and mules (89/148; 60.1%)	*Amblyomma sculptum* (78; F/M) *Dermacentor nitens* (821; F/M/N) *Rhipicephalus microplus* (2; M)	Brazil	Muraro et al., 2021
Privately owned (115/321; 35.8%)	*Hyalomma anatolicum* (1,124) *Rhipicephalus microplus* (813) *Hyalomma dromedarii* (284) *Rhipicephalus haemaphysaloides* (117) *Haemaphysalis bispinosa* (91)	Pakistan	Kamran et al., 2021
Feral (16/36; 44.4%)	*Haemaphysalis parva* (41; F/M) *Hyalomma excavatum* (7; F/M)	Turkey	Dik et al., 2020

	Dermacentor marginatus (2; F/M)		
Removed during regular veterinary welfare exams (51)	*Haemaphysalis longicornis* (245; F/M)	Korea	Seo et al., 2020
Privately owned kept in stalls, paddocks, and pastures (79/200; 39.5%)	*Amblyomma mixtum* (14; F/M) *Dermacentor nitens* (195; F/M/N)	Cuba	Diaz-Sanchez et al., 2020
Stabled (36)	*Amblyomma* sp. (7; L) *Amblyomma cajennense* s.l. (117; F/M/N) *Amblyomma parvum* (1; F) *Dermacentor nitens* (136; F/M/N) *Rhipicephalus microplus* (3; F/M)	Colombia	Santodomingo et al., 2019

Privately owned kept in stalls, paddocks, and pastures (274/1096; 25.0%)	*Haemaphysalis parva* (477; F/M) *Hyalomma excavatum* (1355; F/M) *Hyalomma marginatum* (924; F/M) *Hyalomma turanicum* (7A) *Hyalomma rufipes* (5A) *Rhipicephalus annulatus* (166; F/M/N/L) *Rhipicephalus turanicus* (322; F/M) *Rhipicephalus bursa* (1; A)	Israel	Tirosh-Levy et al., 2018
Privately owned, pastured during day and stalled at night (monthly prevalence ranged from 0–16/24; 0–66.7%)	*Hyalomma scupense* (71; F/M) *Hyalomma marginatum* (34; F/M) *Hyalomma excavatum* (15; F/M)	Tunisia	Gharbi et al. 2018

Free-range pasture grazing with other animals (891/894; 99.7%[b])	*Rhipicephalus (Boophilus) decoloratus* *Amblyomma hebraeum* *Hyalomma rufipes*	Cameroon	Payne et al., 2017
Privately owned and sent in or collected by veterinarians (42)	*Amblyomma triguttatum triguttatum* (15; F/N) *Haemaphysalis bancrofti* (22; F/M/N) *Haemaphysalis longicornis* (207; F/N) *Ixodes holocyclus* (97; F/M/N) *Ixodes tasmani* (1; F) *Rhipicephalus australis* (3; F/N)	Australia	Greay et al., 2016
Privately owned on rural and urban farms (240/360; 66.7%)	*Dermacentor nitens* *Amblyomma mixtum* *Rhipicephalus microplus* *Amblyomma parvum* *Amblyomma tenellum* *Dermacentor dissimilis*	Nicaragua	Duttman et al., 2016

	Amblyomma maculatum		
Grazed in jungles/forests (74)	*Amblyomma cajennense* (1227) *Dermacentor nitens* (593) *Rhipicephalus microplus* (10) Unidentified larvae (250, L)	Guatemala	Teglas et al., 2005

[a]Provided when specified in reference
[b]Includes 5% of horses infested or co-infested with lice
Abbreviations: F, female; M, male; N, nymph; L, larva; A, adult; N, number infested; n, number of ticks; nr, not reported

Table 2. Equine tick-borne diseases, associated pathogens, and primary vectors for tick-borne agents transmitted to horses in North America

Disease	**Disease agent**	**Primary tick vector(s)**	**Reference**
Anaplasmosis	*Anaplasma phagocytophilum*	*Ixodes scapularis* *Ixodes pacificus*	Uehlinger et al., 2011
Lyme borreliosis	*Borrelia burgdorferi*	*Ixodes scapularis* *Ixodes pacificus*	Divers et al., 2018
Rocky Mountain spotted fever	*Rickettsia rickettsii* *Rickettsia amblyommatis* *Rickettsia parkeri* Panola Mountain *Ehrlichia*	*Amblyomma americanum* *Amblyomma maculatum* *Dermacentor variabilis*	Karpathy et al., 2016 Levin et al., 2017 Paddock, 2015 Duncan et al., 2021
Ehrlichiosis	Novel *Ehrlichia* spp.	*Amblyomma americanum*	Carmichael et al., 2014
Equine piroplasmosis	*Theileria equi* *Babesia caballi*	*Amblyomma cajennense* *Dermacentor nitens* *Dermacentor variabilis*	Knowles et al., 2018 Wise et al., 2014

Table 3. Differences seen in mean number of ticks collected from horses at each property

Ranch	Mean number of ticks
1	0.025^{A}
2	0.141^{A}
3	$0.238^{A,B}$
4	$0.278^{A,B}$
5	$1.950^{B,C}$
6	$3.524^{C,D}$
7	$3.706^{C,D}$
8	4.321^{D}

^{A,B,C,D} Values that do not share a superscript are significantly different from one another ($P < 0.05$).

Table 4. Ticks collected from 88 horses in northeastern Oklahoma by species, stage, and month of collection

Species	Stage	Total	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Amblyomma	F	794	0	0	34	186	255	190	81	45	1	0	2	0
americanum	M	906	0	2	31	289	304	198	56	24	0	2	0	0
	N	266	0	0	1	20	68	57	5	41	73	1	0	0
	L	170	0	0	0	0	0	3	0	154	9	2	2	0
Dermacentor	F	52	5	2	0	1	0	0	0	0	0	0	16	28
albipictus	M	7	2	0	0	0	0	0	0	0	0	0	1	4
	N	12	0	0	0	0	0	0	0	0	0	0	9	3
	L	0	0	0	0	0	0	0	0	0	0	0	0	0
Ixodes	F	371	14	4	21	9	3	0	0	0	0	69	226	25
scapularis	M	126	2	0	4	0	0	0	0	0	0	16	97	7
	N	0	0	0	0	0	0	0	0	0	0	0	0	0
	L	0	0	0	0	0	0	0	0	0	0	0	0	0
Other[1]	F	16	0	0	0	1	3	9	2	1	0	0	0	0
	M	10	0	0	0	0	5	2	2	1	0	0	0	0
	N	1	0	0	0	1	0	0	0	0	0	0	0	0
	L	0	0	0	0	0	0	0	0	0	0	0	0	0
Total		2,731	23	8	91	507	638	459	146	266	83	90	353	67

[1]Other ticks collected from horses included *Amblyomma maculatum* and *Dermacentor variabilis*.

Figure 1. Seasonality of tick species and stages infecting horses, October 2019–March 2021

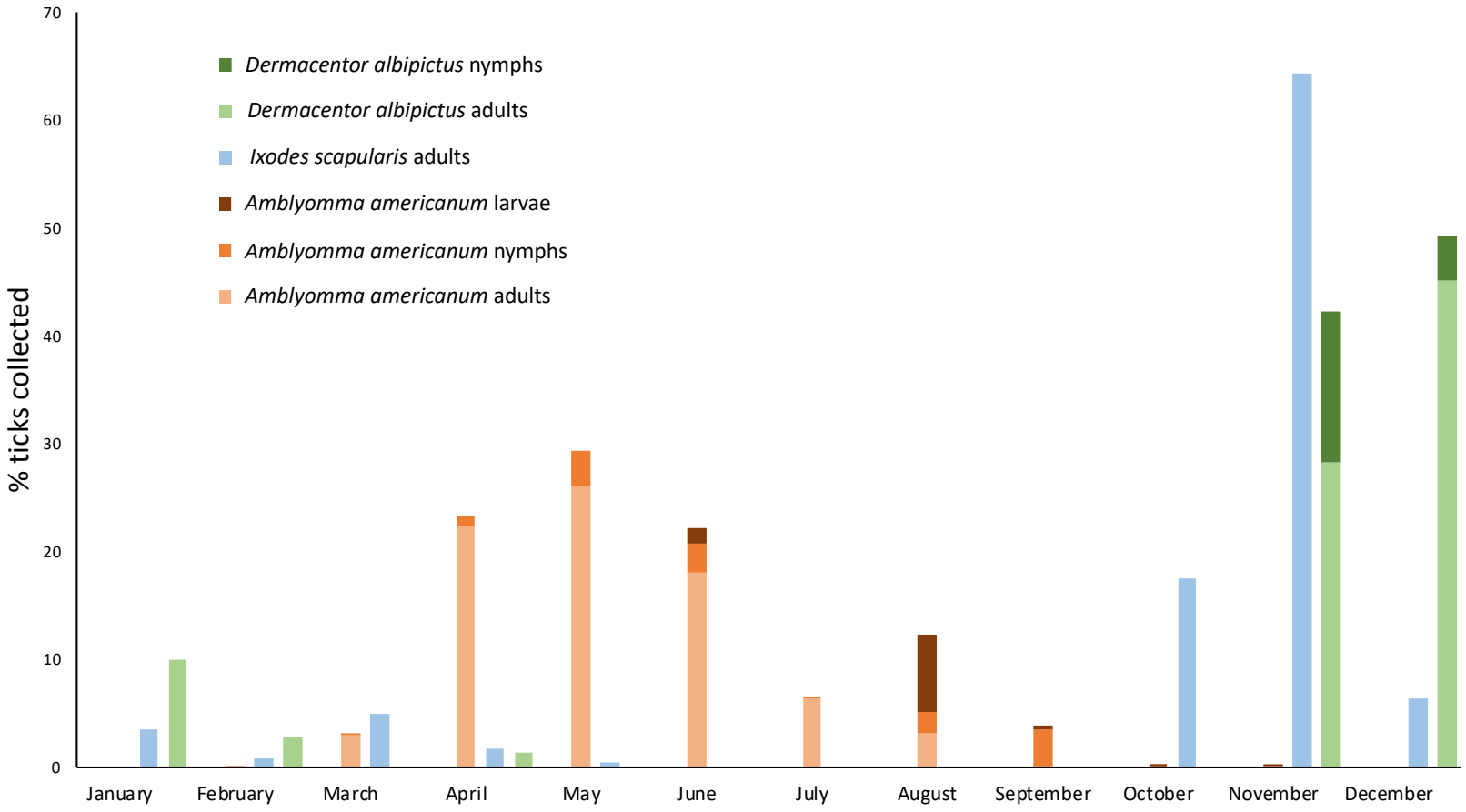
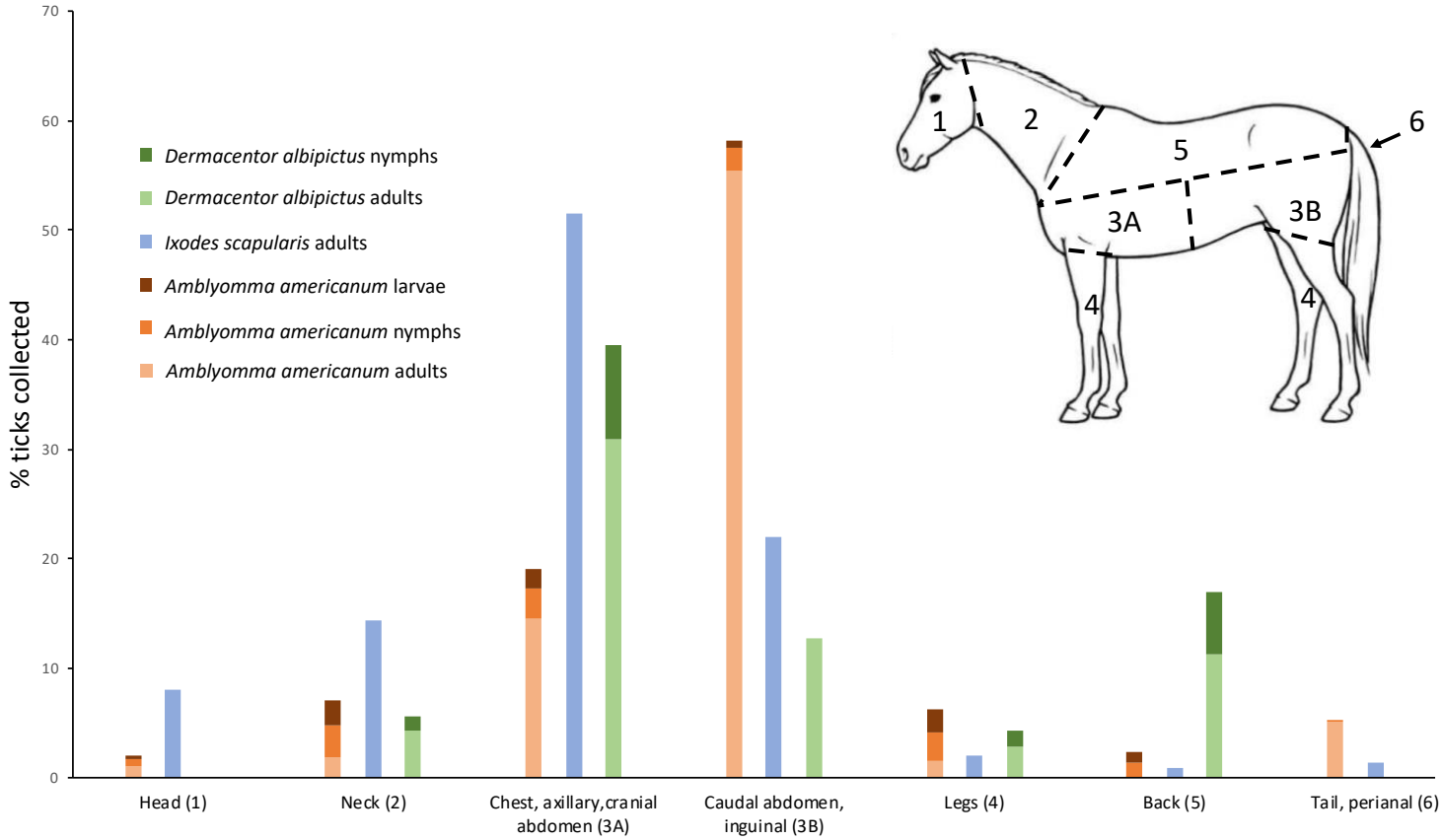


Figure 2. Attachment site preferences of adult *Amblyomma americanum*, *Ixodes scapularis*, and *Dermacentor albipictus* (brown variant) on horses



VITA

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Master of Science

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