COMPARISON OF DIFFERENT SURVEILLANCE METHODS FOR MODELING DISPERSAL OF TICKS

by

TROY MATTHEW KOSER

(Under the Direction of Michael J. Yabsley)

ABSTRACT

Ticks are important vectors for several pathogens and active surveillance through tick collection is an effective way to assess risk to tick-borne disease. In this study we compared the efficiencies of three tick collection methods: tick drags, CO_2-baited drags, and on-host extractions. On-host tick extractions detected seven tick species compared to four species with traditional tick drags and four species with CO_2-baited drags. For the two tick species collected using all methods, on-host extractions collected more *Ixodes scapularis* overall whereas CO_2-baited tick drags collected more *Amblyomma americanum*. On-host tick extractions involved greater time and monetary investment for effective deployment, but focusing on specific wildlife groups makes this technique much more efficient. Pathogen prevalence in environmentally-collected ticks was similar to previous research. Comparing these active tick surveillance methods provides important information for researchers to inform the employment effective collection methods given their interests and resource limitations.

INDEX WORDS: ticks, collection, pathogens, *Amblyomma americanum*, *Ixodes scapularis*

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DEDICATION

I dedicate this thesis to my grandmothers, Judy Nodland and Linda Koser, for enabling my love of nature since I was little. I would also like to dedicate this thesis to my parents, Kari and Dan Koser, who have supported me no matter what path I happen to travel down and to whom I owe a boundless sense of optimism for the future.

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CHAPTER 1

INTRODUCTION

Ticks are important vectors for human and companion animal pathogens and can be serious pests for livestock and wild animal populations. In recent decades the distribution of tick vectors, the composition of their microbial communities, and the number of cases of tick borne illness have changed dramatically (Eisen et al., 2017). Abundance and distribution of specific tick vectors are dictated by a complex relationship between abiotic environmental factors and wildlife host community dynamics. Anthropogenic habitat changes have modified host community compositions and movement patterns while climate change has dramatically affected abiotic environmental factors such as relative humidity, temperature, and soil moisture, affecting and sometimes increasing the abundance and geographic ranges for some medically important ixodid (Family Ixodidae) tick species (Leger et al., 2013; LoGiudice et al., 2003; Sonenshine, 2018). Other important changes to tick ecology have also been noted, such as host associations, seasonal activity periods, and questing behavior differences which can result in shifts in tick community composition, pathogen prevalence, and contact frequency with reservoir hosts (Caminade, 2018; Eisen et al., 2017). Accurately assessing risk to tick-borne pathogens in a given area requires an estimation of tick species distribution, host associations, relative abundance, species-specific questing behaviors, and pathogen prevalence.

Because different tick species have different life histories, they are capable of vectoring a variety of pathogens which makes understanding tick community diversity critical to assessing disease risk in an area. Even differences in questing behavior and host associations within a tick

species can have medically significant impacts on tick-borne pathogen prevalence and risk to infection, as is the case with the Northern and Southern clades of *Ixodes scapularis*. The most common tick-borne disease in the United States is Lyme disease, caused by the spirochete bacteria *Borrelia burgdorferi*, which is vectored mainly by *Ixodes scapularis* (Black legged deer tick) (Stromdahl and Hickling, 2012). This disease is endemic to the Northeastern and Midwestern United States, but not in the Southeastern United States where the tick vector is also present. This is likely due to population-specific differences in questing behavior and host associations which make *Ixodes scpaularis* in the Southeast both less likely to attach to humans and less likely to vector *Borrelia burgdorferi*. Several other tick species do parasitize humans regularly in the Southeast, such as *Amblyomma americanum*, *Amblyomma maculatum*, and *Dermacentor variabilis* (Stromdahl and Hickling, 2012). Determining which species of medically important ticks are in an area, the hosts they may be parasitizing upon, and their pathogen prevalence relies upon effective tick collection methods. The goal of this project is to compare several tick collection techniques and the tick communities they estimate in different habitats in the Southeast. Objectives include the comparison of tick diversity and abundance collected by each method, resource investment in each method, and the determination of prevalence for six veterinary/medically important pathogens in collected tick populations.

LITERATURE CITED

Caminade, C., 2018. Impact of recent and future climate change on vector-borne diseases. Annals of the New York Academy of Sciences.

Eisen, R.J., Kugeler, K.J., Eisen, L., Beard, C.B., Paddock, C.D., 2017. Tick-Borne Zoonoses in the United States: Persistent and Emerging Threats to Human Health. ILAR J 58, 319-335.

Leger, E., Vourc'h, G., Vial, L., Chevillon, C., McCoy, K.D., 2013. Changing distributions of ticks: causes and consequences. Experimental and Applied Acarology 59, 219-244.

LoGiudice, K., Ostfeld, R.S., Schmidt, K.A., Keesing, F., 2003. The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease risk. Proc. Natl. Acad. Sci. U. S. A. 100, 567-571.

Sonenshine, D.E., 2018. Range Expansion of Tick Disease Vectors in North America: Implications for Spread of Tick-Borne Disease. Int. J. Environ. Res. Public Health 15, 9.

Stromdahl, E.Y., Hickling, G.J., 2012. Beyond Lyme: aetiology of tick-borne human diseases with emphasis on the south-eastern United States. Zoonoses Public Health 59 Suppl 2, 48-64.

CHAPTER 2:

LITERATURE REVIEW

TICKS AND THE RISKS THEY POSE TO HUMAN AND ANIMAL HEALTH

Ixodid ticks (Family: Ixodidae), or hard ticks, are major vectors of disease-causing pathogens in humans, companion animals, livestock, and wildlife. Ticks transmit the highest diversity of pathogens of all arthropod vectors and, in the United States, are responsible for 95% of the nationally notifiable human vector-borne disease cases reported to the Centers for Diseases Control and Prevention (CDC) (Parola and Raoult, 2001; Sonenshine, 2018). Beyond their capabilities as vectors, ticks also cause considerable damage to livestock and wildlife when taking blood meals which, given heavy tick infestations, can lead to anemia, malnourishment, and excessive irritation (Glines and Samuel, 1989; Theuret and Trout Fryxell, 2018). Ticks can transmit non-infectious agents as well as infectious pathogens, as is the case with the alpha-gal oligosaccharide which stimulates a sensitivity to red meat consumption in humans (Commins et al., 2011).

Not only are ticks recognized as important influencers in public and veterinary health fields, their importance has recently been described as increasing due to a number of different factors. Climate change has been implicated in spreading the viable habitat range of medically important tick species such as *Amblyomma americanum* (Lone Star tick) and *Amblyomma maculatum* (Gulf Coast tick)(Leger et al., 2013; Sonenshine, 2018). Shifts in habitat structure and composition across the United States have influenced vegetation and wildlife community organization which has, in turn, shifted tick abundance, distribution, and pathogen prevalence.

This has been shown repeatedly through community ecology studies on *Ixodes scapularis* (Black legged deer tick) in the Northeast and Midwest of the United States and a major pathogen of human and animal importance: *Borrelia burgdorferi* (causative agent for Lyme disease)(Blaustein et al., 2010; LoGiudice et al., 2003). Because ticks are able to attach firmly to hosts for relatively long periods of time, they can be effectively transported by animal hosts around the world. Tick introduction can occur through the natural migration of hosts, as with bird migration and *Ixodes scapularis* (Scott and Durden, 2014; Scott et al., 2017), through movement on human hosts, or through the movement of animals for recreation or commercial reasons. The best example of a recent discovery of an exotic tick in the United States can be seen with *Haemaphysalis longicornis* (Asian long horned tick) on a sheep farm in Hunterdon County, New Jersey in 2017 (Rainey et al., 2018). This tick species has since been found in seven states and in records reaching back as far as 2010, implying that there may have been several introduction events over a long period of time before establishment (Theuret and Trout Fryxell, 2018).

With increased interest and research emphasis on tick-borne pathogens comes a concurrent call for increased surveillance for ticks to understand species range and habitat use patterns as well as pathogen prevalence. Public health officials use environmentally collected data to accurately assess risk of exposure to ticks and tick-borne pathogens in an area. Actively collecting ticks from an environment or wildlife hosts, as compared to passively collecting ticks from the public or various human, companion animal, or wildlife health agencies, still arguably represents the best way to quantify tick abundance, range, and pathogen diversity (Mays et al., 2016b; Reye et al., 2012). These active tick collection techniques vary in the types of tick-related stimuli they produce in order to gather ticks from an environment (Cohnstaedt et al.,

2012; Petersen et al., 2015; Springer et al., 2016). For example, some conventional tick collection techniques involve a researcher dragging, flagging, or sweeping a section of cloth (corduroy, flannel, or other durable fabric) across vegetation to collect actively questing ticks (Chong et al., 2013a; Schulze et al., 1997). This method uses movement stimulus as well as some slight semio-chemical stimulus from the researcher to 'activate' ticks and promote attachment to the cloth. Carbon dioxide-baited traps (CO2 traps) use dry ice usually set upon some platform surrounded by sticky tape to lure ticks to a central location using carbon dioxide as a chemical attractant (Falco and Fish, 1989; Kinzer et al., 1990). Similar traps can be made using tick pheromones as well (Cohnstaedt et al., 2012). Extracting ticks off wildlife hosts is a method for collecting ticks that may be more attracted to specific hosts and their associated stimuli rather than general movement or chemical cues (Hertz et al., 2017; Trout Fryxell et al., 2015). Each of these methods is associated with certain biases that vary the tick community abundance and diversity collected and thus the predicted risk for humans or domestic animals. Understanding these differences in collection method bias is crucial to accurately estimating risk to tick-borne pathogens in an area.

ACTIVE SURVEILLANCE FOR TICKS & TICK COLLECTION METHODS

The explosion in importance of Lyme disease in the United States in the late 1970s and early 1980s lead to the first genuine attempts at comprehensive comparisons of tick collection methods in the United States with specific emphasis on the tick vector: *Ixodes scapularis* (Gray, 1985; Schulze et al., 1997). At first, many tick collection studies reported using more traditional etymological collection techniques like chemical-lure traps and sweep netting, which were not effective at collecting the relatively stationary *Ixodes scapularis* tick. Gray (1985) divided effective tick collection techniques into three major categories: flagging, carbon dioxide-baited

traps, and collection from wildlife hosts. To this day these are the most common techniques used in tick collection. Several other techniques using passive surveillance techniques have also been used to assess risk to ticks and tick borne pathogens (Nelder et al., 2014; Soucy et al., 2018; Tulloch et al., 2017), but these records are limited in the type of information researchers are capable of gathering about tick distribution and pathogen prevalence because they often rely on incomplete, voluntarily committed records. Active surveillance can yield information on tick distribution, density, host-associations, pathogen prevalence, and habitat-use patterns which makes it the preferred method for assessing risk to ticks and tick borne pathogens in an area. Few studies have compared collection methods across multiple species and life stages (Mays et al., 2016a; Petry et al., 2010; Rynkiewicz and Clay, 2014b).

Dragging, Flagging, and Sweep Netting

Dragging and flagging have been effective techniques for collecting the nymphal and adult stages of *Ixodes scapularis* in the Northeast for decades. Relatively simple and inexpensive, these techniques can be carried out by a single researcher who drags or flags a section of durable cloth, typically flannel or corduroy, across vegetation that is likely to have the target tick species present. Dragging typically involves using a 1 meter by 1 meter section of cloth attached to a wooden dowel with a length of rope fastened to both ends which the researcher physically drags across low-lying vegetation. For vegetation that may be higher or for tick species that quest further away from the soil, flagging may be used which involves a section of cloth being fastened to a wooden dowel with a section left bare for the researcher to grasp. The researcher then ‘flags’ across waist-to-chest high vegetation. Rulison et al. (2013) found that, for *Ixodes scapularis* collection, these methods are similar in efficiency and thus may be

used interchangeably, but several studies investigating other tick species have assumed this to be true without quantitative studies (Portugal and Goddard, 2015; Tack et al., 2011).

For many medically important tick species across the United States and the world, these techniques have proven effective at collecting sufficient individuals to study aspects of their populations like habitat use, abundance, distribution, and pathogen prevalence. This has been the case for the two most important human-pathogen tick vectors in the United States: *Ixodes scapularis* and *Amblyomma americanum* (Dantas-Torres et al., 2013; Falco and Fish, 1992; Ginsberg and Ewing, 1989; Solberg et al., 1992a). Both ticks have been actively collected by researchers with a dragging or flagging method since at least the 1950s, though recent studies have suggested that even the relatively high number of individuals collected with these techniques may not accurately represent the population of ticks as a whole (Baker, 1952; McCoy et al., 2013). Ticks collected by these methods may, hypothetically, belong to a subpopulation of ticks that respond to more generalized stimuli and may have different pathogen loads than other subpopulations more closely associated with specific hosts (McCoy et al., 2013). Even for known subpopulations of these two species there are some incongruences in collection method efficacy, as seen in the fact that there is no current environmental sampling technique which reliably collects *Ixodes scapularis* nymphs in the Southeast (Tietjen et al., 2019). To be clear, *Ixodes scapularis* nymphs can be found on hosts and the adult stage can be found using the drag technique in the Southeast, but researchers have yet to use or develop an effective environmental collection method for the *Ixodes scapularis* nymph in the Southeast.

The inadequacies of dragging has been noted for several different tick species and communities (Daniels et al., 2000; Dantas-Torres et al., 2013; Dobson, 2013; Li and Dunley, 1998; Rulison et al., 2013; Rynkiewicz and Clay, 2014a). It is well known that several important

tick vectors like *Dermacentor variabilis*, *Amblyomma maculatum*, and *Ixodes pacificus* can be difficult to collect via dragging in flagging in various habitats, especially at the immature stages. Different versions of these techniques like 'swabbing' animal burrows with cloth fashioned to resemble a large cotton swab and weighing down the end of a drag to increase contact rates with soil and leaf litter (where many tick species are presumed to spend the majority of their questing time) have been used to collect endophilic (living inside host burrows) or nidicolous (spend all life stages in nest or burrow) tick species or stages (Cohnstaedt et al., 2012; Portugal and Goddard, 2015). Although dragging and flagging can be used in a variety of different habitats and with relatively few resources, many vegetation types like briar, multiflora rose, and other dense, thorny underbrush plants make these techniques near impossible to execute. Some studies have found significant researcher-linked bias in sampled tick populations, implying that specific walking techniques and personal semiochemicals may play a role in affecting the number of ticks collected in a drag or flag transect (Schulze et al., 1997). These problems affect the reproducibility of these techniques across several seasons and a variety of habitats, which has repeatedly been reported as a major issue in dragging and flagging studies (as well as other tick collection studies) (Dobson, 2013; Hornok et al., 2017).

Chemical Lures and Traps

The use of chemical lures to attract ticks to a specific location has been preferred method for many acarologists because it is relatively inexpensive, requires little time in the field, and can collect ticks in vegetation that may be more difficult to drag or flag. As early as at least the 1960s, remote traps using dry ice bait have been deployed to collect ticks like *Amblyomma americanum* and also *Dermacentor* spp. to study the Colorado Tick Fever virus (Garcia, 1965; Grothaus et al., 1976; Miles, 1968; Wilson et al., 1972). Typical chemical-baited tick traps

involving the use of dry ice (frozen carbon dioxide) are meant to simulate the carbon dioxide gradient in the environment that is produced by the presence of a respiring host or group of hosts to lure ticks to a certain location for extraction. Some traps can be as simple as placing dry ice on a section of white cloth and checking for ticks at specific time intervals (Mays et al., 2016a) while others may involve double-sided tape on a piece of wood or cardboard to hold ticks in place even after the dry ice has completely sublimated (Petry et al., 2010). Even more elaborate mechanisms have been constructed like columns of funnels with vertical climbing platforms or compressed CO2 canisters which slowly release carbon dioxide into the environment more similarly to how a respiring host might be sensed (Carroll, 1988; Springer et al., 2016; Springer et al., 2015). Chemical pheromones have also been used to lure ticks with middling success (Cohnstaedt et al., 2012), but the cost-effectiveness, easy access, and simple deployment of dry ice have made it a common tick lure.

Like flagging and dragging, chemical-baited traps are also imperfect in their ability to sample entire tick populations. Several studies have shown that all tick species and stages are not equally attracted by dry ice traps (Kensinger and Allan, 2011; Mays et al., 2016a; Petry et al., 2010; Solberg et al., 1992b), which collect adult and nymphal *Amblyomma americanum* ticks but rarely pick up *Ixodes scapularis* or *Dermacentor variabilis*. Environmental variables such as wind speed, temperature, and relative humidity affect sublimation rates and concurrent efficiency of dry ice traps (Cohnstaedt et al., 2012; Springer et al., 2015) and the inability to assess the exact range from which ticks might be attracted makes density estimation for tick populations using dry ice trap surveillance inaccurate.

Combination Techniques

In the past decade several studies have proposed novel tick collection techniques which use a combination of stimuli to collect the greatest diversity and abundance of ticks possible using a single collection method. Gherman et al. (2012) used a combination of dry ice and a drag and flag cloth to simulate both a movement and chemical attractant stimulus. The apparatus constructed consisted of a length of pipe threaded with a section of hose with small holes punched into it. The hose is connected to a compressed CO2 canister and the drag cloth is attached to the pipe. These researchers found that the combinatorial collection technique collected more *Ixodes ricinus* and *Dermacentor marginatus* than the conventional flagging and dragging techniques, while Mays et al. (2016a) found that the same technique employed in Tennessee collected more *Amblyomma americanum* than other techniques in some months and collected the only *Ixodes scapularis* nymphs they were able to find, but overall did not collect a greater number of ticks than the conventional dry ice or drag techniques alone.

Although the efficiency of this hybrid technique when compared to conventional techniques has been mixed, the theory behind using multiple stimuli to collect sufficient diversity and abundance of ticks to accurately represent tick communities for risk assessment remains an attractive prospect for tick surveyors. The 'dry ice drag' or 'CO2 drag' (or conversely flag) techniques give researchers the ability to use some form of density measure to assess risk and extrapolate abundances across an area while using two forms of tick stimulus. The equipment required to conduct this collection method is markedly more expensive and technically more difficult to operate than the conventional methods, but materials are still available in most lab settings.

Wildlife Host Tick Extractions

Another common technique to determine species presence and abundance in an area is through sampling wildlife hosts. For many medically important tick species and stages, such as the immature stages of *Amblyomma maculatum* and *Ixodes scapularis*, extracting from wildlife hosts may be the only reliable technique for collecting ticks from the environment. Many game species in the United States, such as the large game species: *Odocoileus virginianus*, *Cervus canadensis*, *Alces* alces, have extensive association histories with their ectoparasite species, often found in the form of opportunistically collected records from harvested animals at hunter check stations (Keefe et al., 2009). Collected ticks from harvested animals can be an efficient method to determine tick distribution and host associations, but only a subset of wildlife can be examined for tick associations in this way and only the harvestable proportion of the game species population would be sampled, thus affecting tick community predictions (Estrada-Pena et al., 2013). Few studies, however, actively survey several wildlife host species specifically for the purposes of extracting and identifying ticks, which can lead to erroneous host associations if conducted incorrectly (Cohnstaedt et al., 2012; Corn et al., 2011; Estrada-Pena et al., 2013; Hamer et al., 2012; Hertz et al., 2017; Merrill et al., 2018; Ogden et al., 2006; Rand et al., 2007). Some animals tend to function as generalists in their environment and are frequent hosts to a number of different tick species and stages, making them ideal sentinels for tick distribution and abundance. Some proposed sentinels in the Southeast include white-tailed deer (*Odocoileus virginianus*), raccoons (*Procyon lotor*), Virginia opossums (*Didelphis virginiana*), and wild hogs (*Sus scrofa*) (Lee et al., 2013; Levi et al., 2012; Merrill et al., 2018; Ouellette et al., 1997). Game animals and nuisance species harvested regularly for various reasons are easier targets for active surveillance studies because they are abundant, their trapping methods are well-

understood, and they can be relatively easy animals to approve for scientific research permits. Birds and rodents are also important hosts whose movement patterns and demographics have profound effects on tick distribution and abundance (Blaustein et al., 2010; Hamer et al., 2012). Wildlife tick collection in general is less reliant on environmental variables such as weather and temperature than other tick collection methods, making it a desirable collection method in extreme or variable environments. It is difficult, however, to extrapolate host infestation data across the host's range without detailed information about host distribution and habitat use, which may not be available.

Some host-tick sampling methods involve surveying domestic animals such as dogs (*Canis familiarus*) and cattle (*Bos taurus, Bos indicus*), but the widespread use of acaricides and complicated task of relating data to other locations makes this technique less than ideal for determining risk to ticks and tick borne pathogens (Abdullah et al., 2016; Theuret and Trout Fryxell, 2018). No matter what host population may be sampled, pathogen prevalence information is more problematically obtained from ticks on wildlife hosts (Estrada-Pena et al., 2013). Partially-fed ticks will contain the blood from their host which may have a suite of pathogens therein that the tick may or may not be able to vector competently from the next stage. Additionally, tick distribution and abundance can be grossly over or under-estimated by host surveillance studies because tick movement does not necessarily result in population establishment and host infestation rates can vary wildly, requiring large-scale and seasonally repeated surveillance events to obtain reliable estimates of prevalence of infestation.

SELECTED TICK SPECIES AND PATHOGENS IN THE SOUTHEAST

The five species most likely to bite and transmit a pathogen to humans in the Southeast are *Amblyomma americanum* (Lone star tick), *Amblyomma maculatum* (Gulf Coast tick),

Dermacentor variabilis (American dog tick), *Ixodes scapularis* (Blacklegged tick), and *Rhipicephalus sanguineus* (Brown dog tick) (Stromdahl and Hickling, 2012). *Amblyomma americanum, Amblyomma maculatum, Dermacentor variabilis,* and *Ixodes scapularis* were collected in this study. Other species of tick found in the Southeast and collected in this study, such as *Ixodes affinis, Ixodes texanus, Haemaphysalis leporispalustris,* and *Ixodes muris*, are less likely to bite humans but may play important roles in the enzootic life cycles of certain pathogens.

Amblyomma americanum

Amblyomma americanum, named the 'Lone Star tick' due to a distinctive white spot on the posterior portion of the scutum on the adult female, is the most common tick species encountered in the Southeast and is known for its aggressive, generalist biting habits (Childs and Paddock, 2003). First described in 1754, *A. americanum* was considered only a nuisance species that commonly attached to livestock, humans, and companion animals in relatively high numbers, but posed no serious concern to medical or veterinary health because no pathogens were associated with the species. Some of the earliest evidence incriminating *A. americanum* as a pathogen vector in human disease cases came from major outbreaks of acute febrile illness in areas with high concentrations of *A. americanum*, as occurred in 1942 in Camp Bullis, Texas (Murray and Dooley, 2004). It was not until the early 1990s, however, when several pathogens associated with illness in humans and animals were isolated from *A. americanum*, that this common tick species came to prominence. In Georgia the adult *A. americanum* is most active in the spring while the nymphal stage has two population peaks in the spring and early fall (Gleim et al., 2014). *A. americanum* associations in Georgia have not been updated in recent surveys, but neighboring or nearby states (Tennessee and Florida) have found all *A. americanum* life

stages on raccoons, opossums, black bears, coyotes, feral swine, white-tailed deer, wild turkey, grey fox, eastern cottontail rabbits, marsh rabbits, and red fox (Cohen et al., 2010a; Hertz et al., 2017). Passerine birds are frequent hosts for immature *A. americanum* and large rodents such as cotton rats and eastern wood rats have been reported as having *A. americanum* infestations, though several recent studies in the Southeast have not found *A. americanum* on rodents (Clark et al., 2002; Clark et al., 2001; Durden et al., 1997; Keirans and Durden, 1998).

The pathogens that first made *A. americanum* a notable threat to public health belong to the *Ehrlichia* genus of bacteria and have been associated with disease cases in humans, companion animals, and livestock (Anderson et al., 1992b; Carmichael et al., 2014; Loftis et al., 2008; Paddock and Childs, 2003). Of particular note, the pathogens *Ehrlichia chaffeensis, Ehrlichia ewingii,* and Panola Mountain *Ehrlichia* have caused disease cases in humans and are primarily transmitted by *A. americanum* (Loftis et al., 2008; Paddock and Childs, 2003; Paddock and Yabsley, 2007). Another bacteria genus, *Rickettsia*, was also important in establishing the pathogen-transmitting capabilities of *A. americanum*. *Rickettsia rickettsii*, the causative agent of Rocky Mountain Spotted Fever (RMSF), was long associated with *Dermacentor variabilis* in the Western United States but thought not to be a disease of public health concern in the Eastern United States (Goddard and Varela-Stokes, 2009). Some studies reporting this pathogen in *A. americanum* in Oklahoma brought discipline focus to the species and fueled several studies on *A. americanum* vectorial capacity for *R. rickettsii* as well as many other tickborne disease pathogens, elucidating important information about *A. americanum* and its microbial community (Goddard and Varela-Stokes, 2009). Although several studies have called into question *A. americanum*'s ability to vector the etiologic agent of RMSF, the quest for *R. rickettsii* in *A. americanum* has found several other *Rickettsia* species such as *Rickettsia amblyommatis* and

Rickettsia parkeri, which may play important roles in causing *Rickettsia*-associated febrile illnesses in people across the Southeast (Stromdahl and Hickling, 2012).

Another tickborne disease associated with *A. americanum* is tularemia, caused by the bacteria *Francisella tularensis*. Since as early as 1949 this disease has been associated with tick bites, but quantifying the proportion of tularemia cases attributable to tick bites versus other forms of transmission (contact with infected animal tissues) and determining the vectorial capacity of different tick species took nearly a decade of additional research (Calhoun et al., 1956; Washburn and Tuohy, 1949). Several studies have found that *F. tularensis* prevalence decreases across *A. americanum* maturation stages and experiments have determined that *F. tularensis* concentrations also decrease across instars, raising questions about transovarial transmission, which has yet to be experimentally established (Petersen et al., 2009).

Though *Borrelia burgdorferi* may be the most famous bacteria of this genus, other *Borrelia* species do exist and are known to cause disease in humans and animals (Boulanger et al., 2019). The discovery of a novel *Borrelia* sp. in *A. americanum* in 1996 linked *A. americanum* with an emerging tickborne disease in the Southeast known as STARI: Southern Tick-Associated Rash Illness (Barbour et al., 1996; Childs and Paddock, 2003). Dubbed *Borrelia lonestari* after its vector, this spirochete has been purported to cause the Lyme-like STARI disease though little evidence has been produced to define this etiological link (Goddard and Varela-Stokes, 2009).

On top of their ability to transmit a diverse array of bacterial pathogens, several viruses associated with diseases in humans have also been isolated from *A. americanum*. The tick-associated acute leukopenia and thrombocytopenia of two farmers in Missouri in 2009 was at first assumed to be the result of an acute *Ehrlichia chaffeensis* infection but later revealed the

discovery of a novel *Phlebovirus*: Heartland virus (HRTV) (Brault et al., 2018; Vasconcelos and Calisher, 2016). The subsequent detection of HRTV in field-collected *A. americanum* adults solidified its association with this tick species while additional studies on vertebrate wildlife neutralizing antibodies to HRTV demonstrated widespread distribution in the central and eastern United States (Riemersma and Komar, 2015; Savage et al., 2016). Since 2009, HRTV disease cases in humans have been reported in the states of Oklahoma, Arkansas, Kansas, Indiana, Kentucky, Tennessee, Georgia, and South Carolina, mainly mirroring the predominant distribution of *A. americanum* across the American Midwest and Southeast (Brault et al., 2018).

Another novel arthropod-borne virus was discovered to be associated with *A. americanum* in 2015 in Bourbon County, Kansas. Bourbon virus (BRBV) belongs to Genus *Thogotovirus* and was the first virus of this genus to be identified in the Western hemisphere (Savage et al., 2018a). BRBV became infamous for causing severe illness and subsequent death in a single human case in 2014, spurring surveillance studies on ticks in the area that revealed nymphal and adult *A. americanum* carrying BRBV (Savage et al., 2018a; Savage et al., 2018b). Field collections of *A. americanum* in eastern Kansas in 2016 and Missouri (following a case of human disease) detected BRBV once more and, surprisingly, surveillance for tickborne viruses in Ehime, Japan in 2016 revealed a *Thogotovirus* closely related to BRBV isolated from *A. testudinarium* ticks (Ejiri et al., 2018; Lambert et al., 2015; Savage et al., 2017; Savage et al., 2018b). Reports of human cases have not spread beyond Kansas, Oklahoma, and Missouri, but the extent of this virus is poorly understood as compared to HRTV (Vasconcelos and Calisher, 2016).

Interestingly, *A. americanum* may be most infamous not for the diversity of bacterial pathogens it can vector nor the potentially fatal viruses it can transmit, but for a non-infectious

agent it can transmit to humans that triggers delayed anaphylaxis response to red meat consumption. Spontaneous development of IgE antibodies to the oligosaccharide galactose-α-1,3-galactose (alpha-gal), which is found in red meat, has been reported in sporadic cases around the United States since 2005, but strangely seemed limited to the Southeast and specifically Tennessee, North Carolina, Arkansas, Virginia, and the southern half of Missouri (Commins et al., 2011). In 2009, studies on this immune sensitivity and its association to patients with tick bite histories and areas with high *A. americanum* abundances revealed a strong correlation between *A. americanum* presence and the development of the red meat allergy as well as a strong correlation between IgE antibodies to alpha-gal and IgE antibodies to *A. americanum* salivary proteins (Commins et al., 2011; Wagner et al., 2012). Further research on mice definitively linked *A. americanum*-derived proteins and the development of an IgE response to alpha-gal (Choudhary et al., 2019). Since the definition of this link, the alpha-gal phenomenon has been a frequent topic for news coverage, which often depict *A. americanum* as causing a 'reverse zombie' allergy (Fulton, 2017). Although the response to this specific aspect of *A. americanum*'s public health importance has often been overinflated and misinformed, it does highlight the interesting complexity of *A. americanum* and its capability to vector an incredible diversity of pathogens and important allergens.

Dog and cat tickborne pathogens are also a concern when it comes to *A. americanum*, which can transmit *Ehrlichia canis* as well as *E. chaffeensis* and *E. ewingii*, causing mild but often chronic infections in dogs (Little et al., 2014). A similar story can be seen with cats in the United States, which can suffer from *A. americanum*-borne *Anaplasma* and *Ehrlichia* species.

A. americanum abundance and distribution have shifted dramatically in the past decade, making large shifts northward into Canada and westward toward Colorado and Nebraska (Barrett

et al., 2015; Childs and Paddock, 2003). Additionally, the pathogen-transmitting abilities of *A. americanum* are just now becoming evident through the discovery in the past decade of several novel bacterial and viral pathogens, making *A. americanum* a significant threat to public health and an important species to survey for distribution, abundance, and pathogen prevalence (Childs and Paddock, 2003; Goddard and Varela-Stokes, 2009).

Because of its generalist questing habits, *A. americanum* has been easily collected using a variety of methods, including flagging or dragging, dry ice traps, and extraction from wildlife hosts (Mays et al., 2016a; Petry et al., 2010; Schulze et al., 2011; Springer et al., 2015). Each collection comparison study which used dry ice as a chemical lur e showed that this method collects a significantly higher number of nymphs and most demonstrated that dry ice methods collect more adults. Collecting *A. americanum* off wildlife has proven an important research objective for determining the routes of invasion through which this generalist tick species may invade new areas, as has been shown repeatedly with bird migrations northward into Canada (de la Fuente et al., 2015; Hamer et al., 2012; Nelder et al., 2019).

Amblyomma maculatum

Another important member of the *Amblyomma* genus in the Southeast is *Amblyomma maculatum*: the Gulf Coast tick. Although one of the more aggressive tick species in the Southeast, *Amblyomma maculatum* was only considered to potentially be of import to animal or human health when renowned microbiologist Ralph R. Parker isolated a novel *Rickettsia* sp., *Rickettsia parkeri*, from *A. maculatum* in southeastern Texas in 1937 (Paddock et al., 2010; Parker et al., 1939; Teel et al., 2010). It was not until 2004, however, that this association was related to a human disease case (Paddock et al., 2010). Now *A. maculatum* is considered a significant threat to human and animal health due to a number of different pathogens it can

vector as well as evidence that its geographical range has been increasing (Stromdahl and Hickling, 2012; Trout et al., 2010). As its name suggests, *A. maculatum*'s original geographic range was thought to be restricted to the Gulf Coast and humidity was once believed to be an important limiting factor to its spread, but recent studies have found that *A. maculatum* can be the dominant tick species in open, dry grasslands and in burned habitats and its populations have extended along the Atlantic coast up to Maryland and inland to Illinois, Kansas, Oklahoma, and even southern Arizona (with isolated expansion events recorded in Maine, Iowa, and New York) (Gleim et al., 2014; Sonenshine, 2018). Common hosts for *A. maculatum* immature stages include rodents and birds while adult stages prefer large mammals such as coyotes, white-tailed deer, feral hogs, black bear, grey fox, red fox, and raccoons (Clark et al., 2001; de la Fuente et al., 2015; Teel et al., 2010).

Since the first confirmation of *Rickettsia parkeri*-associated disease in humans in 2004, several studies have shown increases in the number of reported human disease cases and spread in the geographical distribution of these cases (Paddock et al., 2010; Sonenshine, 2018; Sumner et al., 2007). Belonging to a group of rickettsial bacteria that cause spotted fever group rickettsioses (SFGR), *Rickettsia parkeri* is recognized as an emerging zoonotic pathogen of increasing public health concern because of its relatively high prevalence in some *A. maculatum* populations and because its vector range has been increasing and its seasonality has begun to shift in response to climate change (Paddock et al., 2010; Sonenshine, 2018). Additionally, research on co-feeding of *A. americanum* and *A. maculatum* has shown that this rickettsial pathogen could spillover into the much more generalist and widespread Lone Star tick populations and that it is transtadially and transovarially transmitted in its main *A. maculatum* vector (Wright et al., 2015a; Wright et al., 2015b). Another human disease-causing pathogen

transmitted by *A. maculatum* is Panola Mountain *Ehrlichia*, which was initially believed to be a *A. americanum*-specific pathogen, thus further highlighting potential microbial community synergies between populations of *A. americanum* and *A. maculatum*.

As well as serving as an important vector for human disease-causing pathogens, *A. maculatum* also vectors several pathogens of veterinary medicine importance. *A. maculatum* is considered to be the principle vector for *Hepatozoon americanum*; a protozoal pathogen which causes American canine hepatozoonosis (although *Rhipicephalus sanguineus* is also an important vector) (Ewing and Panciera, 2003; Teel et al., 2010). A significant threat to dogs in the United States, canine hepatozoonosis is considered an emerging disease in the Southeast and is transmitted to dogs when they ingest an infected *A. maculatum*, likely during routine grooming (Ewing and Panciera, 2003). Additionally, *A. maculatum* can vector *Leptospira pomona*, the causative agent for leptospirosis, and *Ehrlichia ruminantium*, the causative agent for heartwater disease, to livestock and ruminants respectively, making it an economically important threat to the agriculture sector in the United States (Teel et al., 2010; Theuret and Trout Fryxell, 2018).

Collection of *A. maculatum* has a more varied history than that of *A. americanum*. Although also a generalist species, the immature stages of *A. maculatum* seem to be more host-specific in their questing behavior and are therefore not frequently collected via dragging or flagging (Mays et al., 2016b; Portugal and Goddard, 2015; Sumner et al., 2007). Adult *A. maculatum* are commonly collected in surveillance studies from grassland habitats while nymphs and larvae are typically found on rodents and birds (Durden et al., 1997; Teel et al., 1998; Trout et al., 2010).

Dermacentor variabilis

The American Dog tick, *Dermacentor variabilis*, has a complicated history in the public health realm. *D. variabilis* is found throughout the United States, from the Southeast all the way to Canada and up through Idaho and Colorado, with an isolated population located west of the Rocky Mountains in California (Minigan et al., 2018; Stromdahl and Hickling, 2012; Yunik et al., 2015). This tick species was first implicated in the transmission cycle of *Rickettsia rickettsii*; the causative agent of Rocky Mountain spotted fever (RMSF), a serious pathogen of human health concern in the United States. RMSF was first reported in the late 19th century in sporadic disease events in Idaho but was soon recognized in human disease cases throughout several western and central American states (Bishopp and Trembley, 1945; Spencer, 1929). Although *Dermacentor andersoni* was often regarded as the principle vector for *R. rickettsii* in the west, in the eastern United States *D. variabilis* was often referred to as the principle vector of this pathogen (Kakumanu et al., 2018). Several studies on *Rickettsia* spp. diversity in *D. variabilis* and molecular verification of clinically-determined 'RMSF' cases in the Southeast have called into question the true relationship between *Rickettsia rickettsii*, the disease RMSF, and *D. variabilis* (Dantas-Torres, 2007; Hecht et al., 2019; Sonenshine, 2018; Stromdahl and Hickling, 2012). Other pathogens of interest to human health which *D. variabilis* can vector include *Francisella tularensis* (the causative agent for tularemia), *Coxiella burnetti* (the causative agent for Q fever), *Ehrlichia chaffeensis* and *E. ewingii*, as well as other member of the *Rickettsia* genus which cause spotted fevers distinct from RMSF (Stromdahl and Hickling, 2012).

Over recent decades, as the importance of *D. variabilis* in the realm of human health and RMSF cases has diminished, the realization of its importance to livestock health has come to the veterinary community. The isolation of *Anaplasma marginale* from *D. variabilis* demonstrates

this tick's potential role in causing bovine anaplasmosis in the cattle industry around the United States, which makes it an economically important pest (Atif, 2015; Kaufman et al., 2018). A recent study on *Anaplasma marginale* seroprevalence in Georgia beef cattle highlighted the disease's importance to the industry in Georgia and subsequent control for its primary tick vector: *Dermacentor variabilis* (Okafor et al., 2019).

Collecting *D. variabilis* has also been a debated topic in the tick collection community, mostly because the vast range of *D. variabilis* has led to some more regionally-specific host preferences and, conversely, questing habits that make different collection methods more effective in different areas (Garcia, 1965; Li and Dunley, 1998; Yoder et al., 2017). In the western and southeastern United States, *D. variabilis* adults can be collected by dragging in appropriate habitats, but significant numbers for pathogen testing may not be available. Immature stages are typically collected off their wildlife hosts, which include rodents and mesomammals (Cohen et al., 2010a; Durden et al., 1997; Kollars et al., 2000; Mays et al., 2016a). In the Midwest and Northeast, *D. variabilis* adults are generally more aggressive and easier to collect by drag or dry ice trap (Petry et al., 2010).

Ixodes scapularis

Of all the ixodid tick species in the United States, none is more likely to be recognized as a major threat to human health than *Ixodes scapularis*: the Blacklegged tick. The discovery in the mid-1970s that *I. scapularis* transmits spirochete bacteria of the *Borrelia burgdorferi sensu lato* complex, the causative agents of Lyme disease in humans and Lyme borreliosis in dogs and cats, was one of the stimulating factors responsible for the recent explosion in research on the diversity of pathogens transmitted by ixodid ticks (Halsey et al., 2018). The complexity of the Lyme disease story and *Ixodes scapularis* ecology, the host-tick-pathogen relationships in this

system, confounds researchers to this day, not to mention the incredible array of other pathogens that *Ixodes scapularis* has been found capable of vectoring (Caminade et al., 2018; Eisen and Eisen, 2018; Nelder et al., 2016; Ogden and Lindsay, 2016). Associated with many different forest habitats across the United States, *Ixodes scapularis*, like *D. variabilis*, has a generalist habitat and host life strategy that make it a good vector for zoonotic pathogens and may also be responsible for some regional differences in questing behavior and pathogen prevalence depending on host availability (McCoy et al., 2013). The diversity of hosts included within *Ixodes scapularis*'s associations is extensive, but importantly it encompasses birds, rabbits, mesomammals such as raccoons and opossums, rodents, large mammals such as deer and black bear, and reptiles (Logiudice et al., 2008). Deer populations were first implicated in maintaining *Ixodes scapularis* populations and serving as reservoirs for *Borrelia burgdorferi sensu stricto*, but experimental studies on white-tailed deer-*Borrelia* infection and correlative studies on Lyme disease cases and deer populations vs. white-footed mice populations (*Peromyscus leucopus*) demonstrated that white-tailed deer are important hosts for adult *Ixodes scapularis* but white-footed mice populations are ultimately more important predictors for the distribution of human Lyme disease cases on the landscape (Halsey et al., 2018; Levi et al., 2012). The use of birds as a host, as has already been mentioned, also serves as an important invasion route through which *Ixodes scapularis* has been able to spread throughout most of the United States and up into Canada (de la Fuente et al., 2015; Kulkarni et al., 2019).

The scope of Lyme disease as a public health issue is still being studied, but it is clearly the most common vector-borne disease in the United States, with 50,000 cases of locally-acquired Lyme reported annually (and 300,000 estimated) making up more than 70% of the tick-borne disease case reports (Eisen and Eisen, 2018). Two other *Borrelia* species within the

Borrelia burgdorferi sensu lato complex (not *Borrelia burgdorferi sensu stricto*), *B. mayonii* and *B. miyamotoi*, have also been found in *Ixodes scapularis* and have caused disease in humans. In 2016 researchers from the Mayo Clinic described the novel spirochete *Borrelia mayonii* as being present in both *Ixodes scapularis* ticks as well as the blood and synovial fluids of six patients with Lyme disease-type symptoms but with markedly higher spirochete loads and more acute neurological symptoms (Pritt et al., 2016). Although this pathogen's importance in the public health realm is still unfolding, the observed symptoms in the clinical cases associated with infection have made it a potential concern as an emerging pathogen. The spirochete *Borrelia miyaomotoi* was discovered much earlier than *Borrelia mayonii*, but a human case was not reported until 2013 (Wormser and Pritt, 2015). Infection with *Borrelia miyamotoi* has been described as being milder than most Lyme disease cases, with asymptomatic and self-resolving cases more common, but the ability of the *Borrelia miyamotoi* spirochete to be transmitted transovarially and its rapid transmission from tick to host increases its human-transmission potential higher than that of *Borrelia burgdorferi sensu stricto*, making it a significant risk to public health (Wormser et al., 2019).

Beyond just Lyme disease, *Ixodes scapularis* is also capable of vectoring several other pathogens that can cause debilitating diseases in humans, many of which are considered emerging diseases in the United States. The causative agent for human granulocytic anaplasmosis, *Anaplasma phagocyophilum*, has also been isolated from *Ixodes scapularis* and has been identified as an emerging pathogen as has *Ehrlichia muris eauclarensis* (Wormser and Pritt, 2015). Interestingly, before the discovery of *Borrelia burgdorferi* in *Ixodes scapularis*, the first zoonotic pathogen isolated from this tick was the protozoal pathogen: *Babesia microti*, which causes human babesiosis (Nelder et al., 2016). As with *A. americanum*, *Ixodes scapularis*

can also transmit a rather pathogenic arbovirus to humans, which is known as the 'deer tick virus', a genotype of the Powassan virus (POWV Lineage II). Powassan virus was found in *Ixodes scapularis* and in humans in the 1950s, but recent studies have found that case numbers for this virus have been increasing which poses a serious threat to public health because POWV-associated infections can lead to severe encephalitis and even death (Ebel, 2010). *Ixodes scapularis* can also transmit *Bartonella henselae*, the causative agent for cat-scratch fever.

The importance of *Ixodes scapularis* to the veterinary medicine community for its ability to transmit pathogens to companion animals and livestock is often overshadowed by the massive number of human Lyme disease cases associated with the tick every year. Similar to *Borrelia*-infections in humans, *Borrelia burgdorferi sensu lato* spirochetes can also infect dogs and cats and cause significant morbidity in companion animal populations every year (Little et al., 2014). Some species of *Anaplasma* also cause significant disease in dogs, like *Anaplasma platys*, which has been isolated from *Ixodes scapularis*. With livestock, *Theileria* and *Babesia* infections can be serious concerns, specifically with horse and cattle operations (Hall et al., 2013).

Increased surveillance for *Ixodes scapularis* for Lyme disease research has made this tick the major species compared in collection method studies in the United States (Daniels et al., 2000; Falco and Fish, 1991, 1992; Rynkiewicz and Clay, 2014b; Schulze et al., 2011; Schulze et al., 1997). Adult *Ixodes scapularis* have been collected in the Northeast and Midwest, where this species is generally a more aggressive quester, for decades yet in other areas of its range adults can still be collected by drag, but in much lower numbers. This phenomenon, as well as some slight morphological differences, lead to the delineation of the Northeastern/Midwestern ticks into the '*Ixodes dammini*' species whereas other tick populations, namely in the southern United States, were called '*Ixodes scapularis*'. Research of genetic relatedness later revealed that these

tick populations do belong to the same species, but still questing behavior, pathogen prevalence, and host associations differ between the Northern and Southern 'Clades' of *Ixodes scapularis*. In the Northeast and Midwest, immature *Ixodes scapularis* can often be found on rodents and birds, making them good wildlife host populations to sample, whereas common immature hosts in the Southeast include reptiles such as snakes and lizards as well as rodents and birds (LoGiudice et al., 2003). This difference in host associations and questing behavior has been used to explain some of the differences in Lyme disease case distribution across the range of *Ixodes scapularis*, since white-footed mice are reservoirs for *Borrelia* bacteria and reptilian blood is usually *Borrelia*cidal, killing the bacteria before the nymphal or adult stages have the opportunity to pass it to humans. The differences in activity patterns for different regional populations of *Ixodes scapularis* exemplifies the need to study the efficiency of several different collection methods for collecting every life stage of a tick species in a specific area.

Ixodes affinis

Some tick species are worth noting not because they can directly vector a pathogen to humans or companion animals or livestock directly, but because they maintain these pathogens in wildlife populations and specifically within reservoir host populations. *Ixodes affinis* is an example of a tick species present in coastal areas around the Southeast which may not play an important role in transmitting disease-causing pathogens directly to humans, but rather because it is an enzootic tick vector for pathogens of medical and veterinary importance. First described in 1953 then at length in 1965, *Ixodes affinis* was not considered by many tick researchers to be a hugely important tick species because it was infrequently reported, found on few wildlife species, and had a fairly limited geographic range in the United States (Gerrish and Ossorio, 1965; Kohls and Rogers, 1953; Oliver et al., 1987). Original range was expected to be along the

southeastern-central Atlantic coast and coastal plain states, from Florida up through North Carolina, with hosts including white-tailed deer and raccoons for adults and birds and rodents for immature stages. Morphologically, *Ixodes affinis* resembles *Ixodes scapularis* and *Ixodes muris* to an extent that most researchers without expert experience in morphological tick identification can rarely distinguish between species, and the fantastic abundance, host variety, and geographic range of *Ixodes scapularis* made it a more commonly identified species.

Since molecular techniques became widely available and tick researchers gained funding for pathogen testing, the differentiation of *Ixodes affinis* became both possible for a lab to verify and also important in distinguishing from *Ixodes scapularis*. Many studies found *Ixodes affinis* much further north, throughout North Carolina and into Virginia, with some sporadic reports further north, including reports from birds migrating into Canada (Harrison et al., 2010; Maggi et al., 2010; Nadolny et al., 2011; Yabsley et al., 2009). Importantly, the hosts included within this tick's associations grew to include several species of mammals and rodents as well as birds, but no reptilian hosts to date (Heller et al., 2016). *Borrelia burgdorferi , B. bissettiae,* and *B. henselae* prevalence in *Ixodes affinis* have repeatedly been much higher than in the human-vector *Ixodes scapularis*, making *Ixodes affinis* likely a critical enzootic vector for *Borrelia* bacteria in the Southeast (Maggi et al., 2019). Several reports of *Ixodes affinis* on dogs and cats have also raised concern about the potential importance of this tick species in companion animal Lyme borreliosis (Hernandez et al., 2015; Nadolny and Gaff, 2018).

The increasing body of evidence that *Ixodes affinis* plays a crucial role in the enzootic of the most important tickborne pathogen in the United States has made its efficient collection a goal for many southeastern tick researchers. Although adult *Ixodes afffinis* can be collected via drag and flag along the Atlantic coastal plain, typically few adults can be gathered in this manner when

compared to *Ixodes scapularis* or *Amblyomma maculatum* (Nadolny and Gaff, 2018). Immature stages of *Ixodes affinis,* however, are not often collected via drag or flag. The relatively recent realization of *Ixodes affinis* as a medically significant species and the noted importance of its wildlife associations in maintaining pathogen enzootic cycles has made it so that studies on *Ixodes affinis* often do include collection via several methods (Nadolny and Gaff, 2018; Nadolny et al., 2011). As the range of this enzootic vector extends further north, into the range of the Northern Clade of *Ixodes scapularis* which are known to bite humans more frequently, fears are that *Borrelia* prevalence in amplifying wildlife hosts will increase and prevalence in *Ixodes scapularis* will increase, raising the likelihood that a human or companion animal will come into contact with an infected tick (Heller et al., 2016). This means *Ixodes affinis* surveillance will be critical in areas with northern *Ixodes scapularis*.

Ixodes texanus

The raccoon tick, *Ixodes texanus*, has a less cryptic history than that of *Ixodes affinis*, because it is fairly distinctive from other *Ixodes* species morphologically and has commonly been found on its namesake host: *Procyon lotor* (raccoon). Originally thought to have a fairly limited number of host associations and small geographic range, *Ixodes texanus* has been found on a number of different hosts and across the mainland United States, from at least Florida up to New York and west to Kansas, and up into Canada and Alaska (Durden et al., 2016; Mock et al., 1991; Rainwater et al., 2017; Smith et al., 2019). Although the raccoon is an important host for nymphal and adult *Ixodes texanus,* they have also been found on numerous mustelid species, some rabbit species, as well as grey and red fox (Brillhart et al., 1994; Gabriel et al., 2009; Lindquist et al., 1999). Studies on the ability of *Ixodes texanus* as an enzootic vector for *Borrelia* or *Ehrlichia* species of bacteria have not shown high prevalences, but more research is likely warranted in this

area given the detection of *Ehrlichia*, *Anaplasma*, and *Borrelia* species bacteria in their host populations (Cohen et al., 2010b; Dugan et al., 2005; Ouellette et al., 1997). As an exclusively wildlife-host tick species, *Ixodes texanus* is an example of a tick that must be collected via extracting ticks from wildlife hosts and the recent discovery of some novel hosts for *Ixodes texanus* highlights the importance of sampling a broad range of hosts even when host associations can appear simple.

Ixodes muris

Alongside *Ixodes affinis*, *Ixodes muris* was also believed to be an important enzootic vector for *Borrelia* spp., mainly because its primary hosts tend to be rodents and birds (Dolan et al., 2000). First described as a rodent-specialist tick species in the Northeast United States & Canada, *Ixodes muris* seemed like a perfect enzootic *Borrelia* vector, but studies on *Borrelia* prevalence and transmission abilities have shown middling vector capabilities compared to *Ixodes scapularis* (Dolan et al., 2000; Scott and Durden, 2015). The discovery of *Ixodes muris* infected with *Borrelia burgdorferi* in Canada, as well as multiple reports of *Ixodes muris* in new state tick records, implicates this tick as an important invasion vector for moving *Borrelia* spp. around North America (Gabriele-Rivet et al., 2015; Scharf and Walker, 2002; Scott and Durden, 2015; Scott et al., 2001). This tick species has also gained notoriety for having an extremely high association between its bites and severe illness in cats and dogs, which has been hypothesized to be due to the host-specific nature of its salivary secretome (Lacombe et al., 1999; Strong-Klefenz and Gaskill, 2008). Studies have called for renewed focus on this underreported tick species to understand its geographic range and host associations. As would be suggested by its host associations, the best techniques for collecting *Ixodes muris* involve sampling its main hosts: songbirds and rodents.

Haemaphysalis leporispalustris

A relatively common tick species, *Haemaphysalis leporispalustris* (the rabbit tick) has long been noted as an important enzootic vector for *Rickettsia* spp., namely *Rickettsia rickettsii*, in the United States but recently little research has been done on this species. The discovery of *Haemaphysalis longicornis* on a sheep farm in Hunterdon County, New Jersey in 2017 and the subsequent realization of this invasive tick's distribution across the United States has brought a renewed focus to *Haemaphysalis* spp. in the US (Egizi et al., 2019; Rainey et al., 2018). *H. leporispalustris* has a wide distribution, from coast-to-coast across the continental United States and has recently been found in Alaska (Durden et al., 2016). Able to competently vector the human pathogens *R. rickettsii* and *Francisella tularensis*, *H. leporispalustris* is considered an important enzootic human pathogen vector as well as a potential invasion vector because of its association with birds at immature stages (Freitas et al., 2009; Hun et al., 2008; Mukherjee et al., 2014; Roth et al., 2017). As its name would suggest, many lagomorph species have host associations with *H. leporispalustris*, but songbird associations with its immature stages are also notable as are some sporadic records of rare host associations on invasive or introduced species such as *Lepus europaeus* and *Antelope cercicapra* (Barros-Battesti and Labruna, 2005; Mertins et al., 1992). These records demonstrate the ability of *H. leporispalustris* to seek out ecological niches on the ectoparasite landscape and has been given as a reason for its wide distribution (Gabriele-Rivet et al., 2015). Collection of *H. leporispalustris* is most commonly achieved through the sampling of passerine birds and rabbit species, although rare associations with larger birds such as turkey and hawks and various larger mammals make them potentially important hosts to sample as well (Lane et al., 2006; Scharf, 2004).

LITERATURE CITED

Abdullah, S., Helps, C., Tasker, S., Newbury, H., Wall, R., 2016. Ticks infesting domestic dogs in the UK: a large-scale surveillance programme. Parasites Vectors 9.

Anderson, B.E., Sumner, J.W., Dawson, J.E., Tzianabos, T., Greene, C.R., Olson, J.G., Fishbein, D.B., Olsenrasmussen, M., Holloway, B.P., George, E.H., Azad, A.F., 1992b. DETECTION OF THE ETIOLOGIC AGENT OF HUMAN EHRLICHIOSIS BY POLYMERASE CHAIN-REACTION. J. Clin. Microbiol. 30, 775-780.

Atif, F.A., 2015. Anaplasma marginale and Anaplasma phagocytophilum: Rickettsiales pathogens of veterinary and public health significance. Parasitol Res 114, 3941-3957.

Baker, E.W., 1952. An introduction to acarology. The Macmillan Company.

Barbour, A.G., Maupin, G.O., Teltow, G.J., Carter, C.J., Piesman, J., 1996. Identification of an uncultivable Borrelia species in the hard tick Amblyomma americanum: Possible agent of a Lyme disease-like illness. J. Infect. Dis. 173, 403-409.

Barrett, A.W., Noden, B.H., Gruntmeir, J.M., Holland, T., Mitcham, J.R., Martin, J.E., Johnson, E.M., Little, S.E., 2015. County Scale Distribution of Amblyomma americanum (Ixodida: Ixodidae) in Oklahoma: Addressing Local Deficits in Tick Maps Based on Passive Reporting. J Med Entomol 52, 269-273.

Barros-Battesti, D.M., Labruna, M.B., 2005. First record of Haemaphysalis leporispalustris (Acari : Ixodidae) on Lepus europaeus (Lagomorpha : Leporidae), an introduced host into the new world. Entomological News 116, 266-267.

Bishopp, F.C., Trembley, H.L., 1945. Distribution and Hosts of Certain North American Ticks. The Journal of parasitology 31, 1-54.

Blaustein, L., Ostfeld, R.S., Holt, R.D., 2010. A COMMUNITY-ECOLOGY FRAMEWORK FOR UNDERSTANDING VECTOR AND VECTOR-BORNE DISEASE DYNAMICS. Israel Journal of Ecology & Evolution 56, 251-262.

Boulanger, N., Boyer, P., Talagrand-Reboul, E., Hansmann, Y., 2019. Ticks and tick-borne diseases. Medecine et maladies infectieuses.

Brault, A.C., Savage, H.M., Duggal, N.K., Eisen, R.J., Staples, J.E., 2018. Heartland Virus Epidemiology, Vector Association, and Disease Potential. Viruses-Basel 10, 17.

Brillhart, D.B., Fox, L.B., Upton, S.J., 1994. TICKS (ACARI, IXODIDAE) COLLECTED FROM SMALL AND MEDIUM-SIZED KANSAS MAMMALS. Journal of Medical Entomology 31, 500-504.

Calhoun, E.L., Mohr, C.O., Alford, H.I., Jr., 1956. Dogs and other mammals as hosts of tularemia and of vector ticks in Arkansas. American journal of hygiene 63, 127-135.

Caminade, C., McIntyre, K.M., Jones, A.E., 2018. Impact of recent and future climate change on vector-borne diseases. Ann N Y Acad Sci.

Carmichael, R.C., Duell, J.R., Holbrook, T.C., Herrin, B.H., Leutenegger, C.M., O'Connor, T.P., Little, S.E., 2014. Antibodies reactive to Ehrlichia spp. are common in Oklahoma horses. Vector Borne Zoonotic Dis 14, 552-556.

Carroll, J.F., 1988. SHORT DURATION DRY ICE SAMPLING FOR AMERICAN DOG TICKS (ACARI, IXODIDAE) IN MARYLAND - A COMPARISON WITH DRAGGING. J. Entomol. Sci. 23, 131-135.

Childs, J.E., Paddock, C.D., 2003. The ascendancy of Amblyomma Americanum as a vector of pathogens affecting humans in the United States. Annu. Rev. Entomol. 48, 307-337.

Chong, S.T., Kim, H.C., Lee, I.-Y., Kollars, T.M., Sancho, A.R., Sames, W.J., Klein, T.A., 2013a. Comparison of Dragging and Sweeping Methods for Collecting Ticks and Determining Their Seasonal Distributions for Various Habitats, Gyeonggi Province, Republic of Korea. Journal of Medical Entomology 50, 611-618.

Choudhary, S., Iweala, O.I., Addison, C.T., Commins, S.P., 2019. Tick Salivary Extract Induces Alpha-Gal Allergy in Alpha-Gal Deficient Mice. J. Allergy Clin. Immunol. 143, AB252-AB252.

Clark, K.L., Oliver, J.H., James, A.M., Durden, L.A., Banks, C.W., 2002. Prevalence of Borrelia burgdorferi sensu lato infection among rodents and host-seeking ticks in South Carolina. Journal of Medical Entomology 39, 198-206.

Clark, K.L., Oliver Jr, J.H., Grego, J.M., James, A.M., Durden, L.A., Banks, C.W., 2001. Host Associations of Ticks Parasitizing Rodents Atborrelia Burgdorferienzootic Sites in South Carolina. Journal of Parasitology 87, 1379-1386.

Cohen, S.B., Freye, J.D., Dunlap, B.G., Dunn, J.R., Jones, T.F., Moncayo, A.C., 2010a. Host Associations of <I>Dermacentor</I>, <I>Amblyomma</I>, and <I>Ixodes</I> (Acari: Ixodidae) Ticks in Tennessee. Journal of Medical Entomology 47, 415-420.

Cohen, S.B., Yabsley, M.J., Freye, J.D., Dunlap, B.G., Rowland, M.E., Huang, J., Dunn, J.R., Jones, T.F., Moncayo, A.C., 2010b. Prevalence of Ehrlichia chaffeensis and Ehrlichia ewingii in Ticks from Tennessee. Vector-Borne and Zoonotic Diseases 10, 435-440.

Cohnstaedt, L.W., Rochon, K., Duehl, A.J., Anderson, J.F., Barrera, R., Su, N.Y., Gerry, A.C., Obenauer, P.J., Campbell, J.F., Lysyk, T.J., Allan, S.A., 2012. Arthropod Surveillance

Programs: Basic Components, Strategies, and Analysis. Ann Entomol Soc Am 105, 135-149.

Commins, S.P., James, H.R., Kelly, L.A., Pochan, S.L., Workman, L.J., Perzanowski, M.S., Kocan, K.M., Fahy, J.V., Nganga, L.W., Ronmark, E., Cooper, P.J., Platts-Mills, T.A.E., 2011. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-alpha-1,3-galactose. J. Allergy Clin. Immunol. 127, 1286-U1310.

Corn, J.L., Mertins, J.W., Hanson, B., Snow, S., 2011. First Reports of Ectoparasites Collected From Wild-Caught Exotic Reptiles in Florida: Table 1. Journal of Medical Entomology 48, 94-100.

Daniels, T.J., Falco, R.C., Fish, D., 2000. Estimating Population Size and Drag Sampling Efficiency for the Blacklegged Tick (Acari: Ixodidae). Journal of Medical Entomology 37, 357-363.

Dantas-Torres, F., 2007. Rocky Mountain spotted fever. The Lancet. Infectious diseases 7, 724-732.

Dantas-Torres, F., Lia, R.P., Capelli, G., Otranto, D., 2013. Efficiency of flagging and dragging for tick collection. Exp Appl Acarol 61, 119-127.

de la Fuente, J., Estrada-Pena, A., Cabezas-Cruz, A., Brey, R., 2015. Flying ticks: anciently evolved associations that constitute a risk of infectious disease spread. Parasites Vectors 8, 11.

Dobson, A.D.M., 2013. Ticks in the wrong boxes: assessing error in blanket-drag studies due to occasional sampling. Parasites Vectors 6, 25.

Dolan, M.C., Lacombe, E.H., Piesman, J., 2000. Vector competence of Ixodes muris (Acari : Ixodidae) for Borrelia burgdorferi. Journal of Medical Entomology 37, 766-768.

Dugan, V.G., Gaydos, J.K., Stallknecht, D.E., Little, S.E., Beall, A.D., Mead, D.G., Hurd, C.C., Davidson, W.R., 2005. Detection of Ehrlichia spp. in raccoons (Procyon lotor) from Georgia. Vector-Borne and Zoonotic Diseases 5, 162-171.

Durden, L.A., Banks, C.W., Clark, K.L., Belbey, B.V., Oliver, J.H., 1997. Ectoparasite fauna of the eastern woodrat, Neotoma floridana: Composition, origin, and comparison with ectoparasite faunas of western woodrat species. Journal of Parasitology 83, 374-381.

Durden, L.A., Beckmen, K.B., Gerlach, R.F., 2016. New Records of Ticks (Acari: Ixodidae) From Dogs, Cats, Humans, and Some Wild Vertebrates in Alaska: Invasion Potential. Journal of Medical Entomology 53, 1391-1395.

Ebel, G.D., 2010. Update on Powassan Virus: Emergence of a North American Tick-Borne Flavivirus. Annu. Rev. Entomol. 55, 95-110.

Egizi, A.M., Robbins, R.G., Beati, L., Navas, S., Evans, C.R., Occi, J.L., Fonseca, D.M., 2019. A pictorial key to differentiate the recently detected exotic Haemaphysalis longicornis Neumann, 1901 (Acari, Ixodidae) from native congeners in North America. Zookeys, 117-128.

Eisen, R.J., Eisen, L., 2018. The Blacklegged Tick, Ixodes scapularis: An Increasing Public Health Concern. Trends Parasitol. 34, 295-309.

Ejiri, H., Lim, C.K., Isawa, H., Fujita, R., Murota, K., Sato, T., Kobayashi, D., Kan, M., Hattori, M., Kimura, T., Yamaguchi, Y., Takayama-Ito, M., Horiya, M., Posadas-Herrera, G., Minami, S., Kuwata, R., Shimoda, H., Maeda, K., Katayama, Y., Mizutani, T., Saijo, M., Kaku, K., Shinomiya, H., Sawabe, K., 2018. Characterization of a novel thogotovirus

isolated from Amblyomma testudinarium ticks in Ehime, Japan: A significant phylogenetic relationship to Bourbon virus. Virus Research 249, 57-65.

Estrada-Pena, A., Gray, J.S., Kahl, O., Lane, R.S., Nijhof, A.M., 2013. Research on the ecology of ticks and tick-borne pathogens--methodological principles and caveats. Front Cell Infect Microbiol 3, 29.

Ewing, S.A., Panciera, R.J., 2003. American canine hepatozoonosis. Clinical microbiology reviews 16, 688-697.

Falco, R.C., Fish, D., 1989. THE USE OF CARBON DIOXIDE-BAITED TICK TRAPS FOR SAMPLING IXODES-DAMMINI (ACARI, IXODIDAE). Acarologia 30, 29-33.

Falco, R.C., Fish, D., 1991. HORIZONTAL MOVEMENT OF ADULT IXODES-DAMMINI (ACARI, IXODIDAE) ATTRACTED TO CO2-BAITED TRAPS. Journal of Medical Entomology 28, 726-729.

Falco, R.C., Fish, D., 1992. A COMPARISON OF METHODS FOR SAMPLING THE DEER TICK, IXODES-DAMMINI, IN A LYME-DISEASE ENDEMIC AREA. Exp. Appl. Acarol. 14, 165-173.

Freitas, L.H.T., Faccini, J.L.H., Labruna, M.B., 2009. Experimental infection of the rabbit tick, Haemaphysalis leporispalustris, with the bacterium Rickettsia rickettsii, and comparative biology of infected and uninfected tick lineages. Experimental and Applied Acarology 47, 321-345.

Fulton, J. 2017. The “Reverse Zombie" Tick Is Spreading Around America, Causing A Strange Condition As It Goes (IFLSCIENCE!.com).

Gabriel, M.W., Brown, R.N., Foley, J.E., Higley, J.M., Botzler, R.G., 2009. ECOLOGY OF ANAPLASMA PHAGOCYTOPHILUM INFECTION IN GRAY FOXES (UROCYON

CINEREOARGENTEUS) IN NORTHWESTERN CALIFORNIA. J. Wildl. Dis. 45, 344-354.

Gabriele-Rivet, V., Arsenault, J., Badcock, J., Cheng, A., Edsall, J., Goltz, J., Kennedy, J., Lindsay, L.R., Pelcat, Y., Ogden, N.H., 2015. Different Ecological Niches for Ticks of Public Health Significance in Canada. Plos One 10.

Garcia, R., 1965. COLLECTION OF DERMACENTOR ANDERSONI (STILES) WITH CARBON DIOXIDE AND ITS APPLICATION IN STUDIES OF COLORADO TICK FEVER VIRUS. American Journal of Tropical Medicine and Hygiene 14, 1090-&.

Gerrish, R.R., Ossorio, J.M., 1965. NEW RECORD OF TICK IXODES AFFINIS IN UNITED STATES. Journal of Economic Entomology 58, 369-&.

Gherman, C.M., Mihalca, A.D., Dumitrache, M.O., Gyorke, A., Oroian, I., Sandor, M., Cozma, V., 2012. CO2 flagging - an improved method for the collection of questing ticks. Parasites Vectors 5, 7.

Ginsberg, H.S., Ewing, C.P., 1989. COMPARISON OF FLAGGING, WALKING, TRAPPING, AND COLLECTING FROM HOSTS AS SAMPLING METHODS FOR NORTHERN DEER TICKS, IXODES-DAMMINI, AND LONE-STAR TICKS, AMBLYOMMA-AMERICANUM (ACARI, IXODIDAE). Exp. Appl. Acarol. 7, 313-322.

Gleim, E.R., Conner, L.M., Berghaus, R.D., Levin, M.L., Zemtsova, G.E., Yabsley, M.J., 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, 10.

Glines, M.V., Samuel, W.M., 1989. EFFECT OF DERMACENTOR-ALBIPICTUS (ACARI, IXODIDAE) ON BLOOD COMPOSITION, WEIGHT-GAIN AND HAIR COAT OF MOOSE, ALCES-ALCES. Exp. Appl. Acarol. 6, 197-213.

Goddard, J., Varela-Stokes, A.S., 2009. Role of the lone star tick, Amblyomma americanum (L.), in human and animal diseases. Veterinary Parasitology 160, 1-12.

Gray, J.S., 1985. TICKS - THEIR ECONOMIC IMPORTANCE AND METHODS OF CONTROL. Outlook on Agriculture 14, 136-142.

Grothaus, R.H., Haskins, J.R., Reed, J.T., 1976. SIMPLIFIED CARBON-DIOXIDE COLLECTION TECHNIQUE FOR RECOVERY OF LIVE TICKS (ACARINA). Journal of Medical Entomology 12, 702-702.

Hall, C.M., Busch, J., Scoles, G., Palma-Cagle, K., Ueti, M., 2013. Genetic characterization of Theileria equi infecting horses in North America: evidence for a limited source of U.S. introductions. Parasites and Vectors 6, 12.

Halsey, S.J., Allan, B.F., Miller, J.R., 2018. The role of Ixodes scapularis, Borrelia burgdorferi and wildlife hosts in Lyme disease prevalence: A quantitative review. Ticks Tick-Borne Dis. 9, 1103-1114.

Hamer, S.A., Goldberg, T.L., Kitron, U.D., Brawn, J.D., Anderson, T.K., Loss, S.R., Walker, E.D., Hamer, G.L., 2012. Wild birds and urban ecology of ticks and tick-borne pathogens, Chicago, Illinois, USA, 2005-2010. Emerging infectious diseases 18, 1589-1595.

Harrison, B.A., Rayburn, W.H., Toliver, M., Powell, E.E., Engber, B.R., Durden, L.A., Robbins, R.G., Prendergast, B.F., Whitt, P.B., 2010. Recent discovery of widespread Ixodes affinis (Acari: Ixodidae) distribution in North Carolina with implications for Lyme disease studies. J. Vector Ecol. 35, 174-179.

Hecht, J.A., Allerdice, M.E.J., Dykstra, E.A., Mastel, L., Eisen, R.J., Johnson, T.L., Gaff, H.D., Varela-Stokes, A.S., Goddard, J., Pagac, B.B., Paddock, C.D., Karpathy, S.E., 2019.

Heller, E.L., Wright, C.L., Nadolny, R.M., Hynes, W.L., Gaff, H.D., Walters, E.L., 2016. New Records of Ixodes affinis (Acari: Ixodidae) Parasitizing Avian Hosts in Southeastern Virginia. Journal of Medical Entomology 53, 441-445.

Hernandez, A.S., Vivas, R.I.R., Barrera, M.A.P., Gassent, M.D.E., Apanaskevich, D.A., 2015. Ixodes affinis (Acari: Ixodidae) in dogs from the rural communities of Yucatan, Mexico: prevalence, abundance and associated factors. Veterinaria Mexico 2.

Hertz, J.C., Ferree Clemons, B.C., Lord, C.C., Allan, S.A., Kaufman, P.E., 2017. Distribution and host associations of ixodid ticks collected from wildlife in Florida, USA. Exp Appl Acarol 73, 223-236.

Hornok, S., Meli, M.L., Gonczi, E., Hofmann-Lehmann, R., 2017. SEASONALLY BIASED OR SINGLE-HABITAT SAMPLING IS NOT INFORMATIVE ON THE REAL PREVALENCE OF DERMACENTOR RETICULATUS-BORNE RICKETTSIAE - A PILOT STUDY. Acta Veterinaria Hungarica 65, 81-88.

Hun, L., Cortes, X., Taylor, L., 2008. Molecular Characterization of Rickettsia rickettsii Isolated from Human Clinical Samples and from the Rabbit Tick Haemaphysalis leporispalustris Collected at Different Geographic Zones in Costa Rica. American Journal of Tropical Medicine and Hygiene 79, 899-902.

Kakumanu, M.L., Ponnusamy, L., Sutton, H., Meshnick, S.R., Nicholson, W.L., Apperson, C.S., 2018. Prevalence of Rickettsia Species (Rickettsiales: Rickettsiaceae) in Dermacentor variabilis Ticks (Acari: Ixodidae) in North Carolina. Journal of Medical Entomology 55, 1284-1291.

Kaufman, E.L., Stone, N.E., Scoles, G.A., Hepp, C.M., Busch, J.D., Wagner, D.M., 2018. Range-wide genetic analysis of Dermacentor variabilis and its Francisella-like

endosymbionts demonstrates phylogeographic concordance between both taxa. Parasites Vectors 11, 306-306.

Keefe, L.M., Moro, M.H., Vinasco, J., Hill, C., Wu, C.C., Raizman, E.A., 2009. The Use of Harvested White-Tailed Deer (Odocoileus virginianus) and Geographic Information System (GIS) Methods to Characterize Distribution and Locate Spatial Clusters of Borrelia burgdorferi and Its Vector Ixodes scapularis in Indiana. Vector-Borne and Zoonotic Diseases 9, 671-680.

Keirans, J.E., Durden, L.A., 1998. Illustrated key to nymphs of the tick genus Amblyomma (Acari: Ixodidae) found in the United States. J Med Entomol 35, 489-495.

Kensinger, B.J., Allan, B.F., 2011. Efficacy of Dry Ice-Baited Traps for Sampling Amblyomma americanum (Acari: Ixodidae) Varies With Life Stage But Not Habitat. Journal of Medical Entomology 48, 708-711.

Kinzer, D.R., Presley, S.M., Hair, J.A., 1990. COMPARATIVE EFFICIENCY OF FLAGGING AND CARBON DIOXIDE-BAITED STICKY TRAPS FOR COLLECTING THE LONE STAR TICK, AMBLYOMMA-AMERICANUM (ACARINA, IXODIDAE). Journal of Medical Entomology 27, 750-755.

Kohls, G.M., Rogers, A.J., 1953. NOTE ON THE OCCURRENCE OF THE TICK IXODES-AFFINIS NEUMANN IN THE UNITED-STATES. Journal of Parasitology 39, 669-669.

Kollars, T.M., Oliver, J.H., Masters, E.J., Kollars, P.G., Durden, L.A., 2000. Host utilization and seasonal occurrence of Dermacentor. Exp. Appl. Acarol. 24, 631-643.

Kulkarni, M.A., Narula, I., Slatculescu, A.M., Russell, C., 2019. Lyme Disease Emergence after Invasion of the Blacklegged Tick, Ixodes scapularis, Ontario, Canada, 2010-2016. Emerg Infect Dis 25, 328-332.

Lacombe, E.H., Rand, P.W., Smith, R.P., 1999. Severe reaction in domestic animals following the bite of Ixodes muris (Acari : Ixodidae). Journal of Medical Entomology 36, 227-232.

Lambert, J., Velez, J.O., Brault, A.C., Calvert, A.E., Bell-Sakyi, L., Bosco-Lauth, A.M., Staples, J.E., Kosoya, O.I., 2015. Molecular, serological and in vitro culture-based characterization of Bourbon virus, a newly described human pathogen of the genus Thogotovirus. Journal of Clinical Virology 73, 127-132.

Lane, R.S., Kucera, T.F., Barrett, R.H., Mun, J., Wu, C.L., Smith, V.S., 2006. Wild turkey (Meleagris gallopavo) as a host of ixodid ticks, lice, and lyme disease spirochetes (Borrelia burgdorferi sensu lato) in California state parks. J. Wildl. Dis. 42, 759-771.

Lee, X., Hardy, K., Johnson, D.H., Paskewitz, S.M., 2013. Hunter-Killed Deer Surveillance to Assess Changes in the Prevalence and Distribution of Ixodes scapularis (Acari: Ixodidae) in Wisconsin. Journal of Medical Entomology 50, 632-639.

Leger, E., Vourc'h, G., Vial, L., Chevillon, C., McCoy, K.D., 2013. Changing distributions of ticks: causes and consequences. Experimental and Applied Acarology 59, 219-244.

Levi, T., Kilpatrick, A.M., Mangel, M., Wilmers, C.C., 2012. Deer, predators, and the emergence of Lyme disease. Proc Natl Acad Sci U S A 109, 10942-10947.

Li, X.H., Dunley, J.E., 1998. Optimal sampling and spatial distribution of Ixodes pacificus, Dermacentor occidentalis and Dermacentor variabilis ticks (Acari : Ixodidae). Exp. Appl. Acarol. 22, 233-248.

Lindquist, E.E., Wu, K.W., Redner, J.H., 1999. A new species of the tick genus Ixodes (Acari : Ixodidae) parasitic on mustelids (Mammalia : Carnivora) in Canada. Canadian Entomologist 131, 151-170.

Little, S.E., Beall, M., Bowman, D.D., Chandrashekar, R., Stamaris, J., 2014. Canine infection with Diroflaria immitis, Borrelia burgdorferi, Anaplasma spp., and Erlichia spp. in the United States, 2010-2012. Parasites and Vectors 7, 257.

Loftis, A.D., Mixson, T.R., Stromdahl, E.Y., Yabsley, M.J., Garrison, L.E., Williamson, P.C., Fitak, R.R., Fuerst, P.A., Kelly, D.J., Blount, K.W., 2008. Geographic distribution and genetic diversity of the Ehrlichia sp. from Panola Mountain in Amblyomma americanum. BMC Infect Dis 8, 54.

Logiudice, K., Duerr, S.T.K., Newhouse, M.J., Schmidt, K.A., Killilea, M.E., Ostfeld, R.S., 2008. IMPACT OF HOST COMMUNITY COMPOSITION ON LYME DISEASE RISK. Ecology 89, 2841-2849.

LoGiudice, K., Ostfeld, R.S., Schmidt, K.A., Keesing, F., 2003. The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease risk. Proc. Natl. Acad. Sci. U. S. A. 100, 567-571.

Maggi, R.G., Reichelt, S., Toliver, M., Engber, B., 2010. Borrelia species in Ixodes affinis and Ixodes scapularis ticks collected from the coastal plain of North Carolina. Ticks Tick-Borne Dis. 1, 168-171.

Maggi, R.G., Toliver, M., Richardson, T., Mather, T., Breitschwerdt, E.B., 2019. Regional prevalences of Borrelia burgdorferi, Borrelia bissettiae, and Bartonella henselae in Ixodes affinis, Ixodes pacificus and Ixodes scapularis in the USA. Ticks Tick-Borne Dis. 10, 360-364.

Mays, S.E., Houston, A.E., Fryxell, R.T.T., 2016a. Comparison of novel and conventional methods of trapping ixodid ticks in the southeastern USA. Medical and Veterinary Entomology 30, 123-134.

Mays, S.E., Houston, A.E., Trout Fryxell, R.T., 2016b. Comparison of novel and conventional methods of trapping ixodid ticks in the southeastern U.S.A. Med Vet Entomol 30, 123-134.

McCoy, K.D., Leger, E., Dietrich, M., 2013. Host specialization in ticks and transmission of tick-borne diseases: a review. Front. Cell. Infect. Microbiol. 3, 12.

Merrill, M.M., Boughton, R.K., Lord, C.C., Sayler, K.A., Wight, B., Anderson, W.M., Wisely, S.M., 2018. Wild pigs as sentinels for hard ticks: A case study from south-central Florida. Int. J. Parasitol.-Parasit. Wildl. 7, 161-170.

Mertins, J.W., Schlater, J.L., Corn, J.L., 1992. ECTOPARASITES OF THE BLACKBUCK ANTELOPE (ANTILOPE-CERVICAPRA). J. Wildl. Dis. 28, 481-484.

Miles, V.I., 1968. A CARBON DIOXIDE BAIT TRAP FOR COLLECTING TICKS AND FLEAS FROM ANIMAL BURROWS. Journal of Medical Entomology 5, 491-&.

Minigan, J.N., Hager, H.A., Peregrine, A.S., Newman, J.A., 2018. Current and potential future distribution of the American dog tick (Dermacentor variabilis, Say) in North America. Ticks Tick Borne Dis 9, 354-362.

Mock, D.E., Brillhart, D.B., Fox, L.B., Upton, S.J., 1991. TICKS (ACARI, IXODIDAE) FROM RACCOONS (PROCYON LOTOR) IN EASTERN KANSAS, WITH A NEW STATE RECORD FOR IXODES-TEXANUS. Journal of the Kansas Entomological Society 64, 344-345.

Mukherjee, N., Beati, L., Sellers, M., Burton, L., Adamson, S., Robbins, R.G., Moore, F., Karim, S., 2014. Importation of exotic ticks and tick-borne spotted fever group rickettsiae into the United States by migrating songbirds. Ticks Tick-Borne Dis. 5, 127-134.

Murray, C.K., Dooley, D.P., 2004. Bullis fever: a vanished infection of unknown etiology. Military medicine 169, 863-865.

Nadolny, R.M., Gaff, H.D., 2018. Natural history of Ixodes affinis in Virginia. Ticks Tick-Borne Dis. 9, 109-119.

Nadolny, R.M., Wright, C.L., Hynes, W.L., Sonenshine, D.E., Gaff, H.D., 2011. Ixodes affinis (Acari: Ixodidae) in southeastern Virginia and implications for the spread of Borrelia burgdorferi, the agent of Lyme disease. J. Vector Ecol. 36, 464-467.

Nelder, M.P., Russell, C., Lindsay, L.R., Dhar, B., Patel, S., Johnson, S., 2014. Population-Based Passive Tick Surveillance and Detection of Expanding Foci of Blacklegged Ticks Ixodes scapularis and the Lyme Disease Agent Borrelia burgdorferi in Ontario, Canada. PLOS One 9, 12.

Nelder, M.P., Russell, C.B., Clow, K.M., Johnson, S., Weese, J.S., Cronin, K., Ralevski, F., Jardine, C.M., Patel, S.N., 2019. Occurrence and distribution of Ambylomma americanum as determined by passive surveillance in Ontario, Canada (1999–2016). Ticks Tick-Borne Dis. 10, 146-155.

Nelder, M.P., Russell, C.B., Sheehan, N.J., Sander, B., Moore, S., Li, Y., Johnson, S., Patel, S.N., Sider, D., 2016. Human pathogens associated with the blacklegged tick Ixodes scapularis: a systematic review. Parasites Vectors 9, 14.

Ogden, N.H., Lindsay, L.R., 2016. Effects of Climate and Climate Change on Vectors and Vector-Borne Diseases: Ticks Are Different. Trends Parasitol. 32, 646-656.

Ogden, N.H., Trudel, L., Artsob, H., Barker, I.K., Beauchamp, G., Charron, D.F., Drebot, M.A., Galloway, T.D., O'Handley, R., Thompson, R.A., Lindsay, L.R., 2006. Ixodes scapularis

ticks collected by passive surveillance in Canada: analysis of geographic distribution and infection with Lyme borreliosis agent Borrelia burgdorferi. J Med Entomol 43, 600-609.

Okafor, C.C., Collins, S.L., Daniel, J.A., Coetzee, J.F., Whitlock, B.K., 2019. Seroprevalence of bovine Anaplasmosis in Georgia. Veterinary Parasitology: Regional Studies and Reports 15, 100258.

Oliver, J.H., Keirans, J.E., Lavender, D.R., Hutcheson, H.J., 1987. IXODES-AFFINIS NEUMANN (ACARI, IXODIDAE) - NEW HOST AND DISTRIBUTION RECORDS, DESCRIPTION OF IMMATURES, SEASONAL ACTIVITIES IN GEORGIA, AND LABORATORY REARING. Journal of Parasitology 73, 646-652.

Ouellette, J., Apperson, C.S., Howard, P., Evans, T.L., Levine, J.F., 1997. Tick-raccoon associations and the potential for Lyme disease spirochete transmission in the coastal plain of North Carolina. J Wildl Dis 33, 28-39.

Paddock, C.D., Childs, J.E., 2003. Ehrlichia chaffeensis: a Prototypical Emerging Pathogen. Clinical Microbiology Reviews 16, 37-64.

Paddock, C.D., Fournier, P.E., Sumner, J.W., Goddard, J., Elshenawy, Y., Metcalfe, M.G., Loftis, A.D., Varela-Stokes, A., 2010. Isolation of Rickettsia parkeri and identification of a novel spotted fever group Rickettsia sp. from Gulf Coast ticks (Amblyomma maculatum) in the United States. Appl Environ Microbiol 76, 2689-2696.

Paddock, C.D., Yabsley, M.J., 2007. Ecological havoc, the rise of white-tailed deer, and the emergence of Amblyomma americanum-associated zoonoses in the United States. Current topics in microbiology and immunology 315, 289-324.

Parker, R., Kohls, G.M., Cox, G.W., Davis, G.E., 1939. Observations on an infectious agent from Amblyomma maculatum. Public Health Reports (1896-1970), 1482-1484.

Parola, P., Raoult, D., 2001. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. (vol 32, pg 897, 2001). Clin. Infect. Dis. 33, 749-749.

Petersen, J.M., Mead, P.S., Schriefer, M.E., 2009. Francisella tularensis: an arthropod-borne pathogen. Veterinary research 40, 7.

Petersen, W.H., Foster, E., McWilliams, V., Irwin, W. 2015. Tick-borne Disease Surveillance, Defense, D.o., ed. (The Army Medical Department Journal).

Petry, W.K., Fore, S.A., Fielden, L.J., Kim, H.J., 2010. A quantitative comparison of two sample methods for collecting Amblyomma americanum and Dermacentor variabilis (Acari: Ixodidae) in Missouri. Exp Appl Acarol 52, 427-438.

Portugal, J.S., Goddard, J., 2015. Collections of immature Amblyomma maculatum Koch (Acari: Ixodidae) from Mississippi, USA. Systematic and Applied Acarology 20, 20-24.

Pritt, B.S., Respicio-Kingry, L.B., Sloan, L.M., Schriefer, M.E., Replogle, A.J., Bjork, J., Liu, G.P., Kingry, L.C., Mead, P.S., Neitzel, D.F., Schiffman, E., Johnson, D.K.H., Davis, J.P., Paskewitz, S.M., Boxrud, D., Deedon, A., Lee, X., Miller, T.K., Feist, M.A., Steward, C.R., Thee, E.S., Pate, R., Irish, C.L., Petersen, J.M., 2016. Borrelia mayonii sp nov., a member of the Borrelia burgdorferi sensu lato complex, detected in patients and ticks in the upper midwestern United States. International Journal of Systematic and Evolutionary Microbiology 66, 4878-4880.

Rainey, T., Occi, J.L., Robbins, R.G., Egizi, A., 2018. Discovery of Haemaphysalis longicornis (Ixodida: Ixodidae) Parasitizing a Sheep in New Jersey, United States. Journal of Medical Entomology 55, 757-759.

Rainwater, K.L., Marchese, K., Slavinski, S., Humberg, L.A., Dubovi, E.J., Jarvis, J.A., McAloose, D., Calle, P.P., 2017. HEALTH SURVEY OF FREE-RANGING

RACCOONS (PROCYON LOTOR) IN CENTRAL PARK, NEW YORK, NEW YORK, USA: IMPLICATIONS FOR HUMAN AND DOMESTIC ANIMAL HEALTH. J. Wildl. Dis. 53, 272-284.

Rand, P.W., Lacombe, E.H., Dearborn, R., Cahill, B., Elias, S., Lubelczyk, C.B., Beckett, G.A., Smith, R.P., Jr., 2007. Passive surveillance in Maine, an area emergent for tick-borne diseases. J Med Entomol 44, 1118-1129.

Reye, A.L., Arinola, O.G., Hubschen, J.M., Muller, C.P., 2012. Pathogen prevalence in ticks collected from the vegetation and livestock in Nigeria. Appl Environ Microbiol 78, 2562-2568.

Riemersma, K.K., Komar, N., 2015. Heartland Virus Neutralizing Antibodies in Vertebrate Wildlife, United States, 2009-2014. Emerging Infectious Diseases 21, 1830-1833.

Roth, T., Lane, R.S., Foley, J., 2017. A Molecular Survey for Francisella tularensis and Rickettsia spp. in Haemaphysalis leporispalustris (Acari: Ixodidae) in Northern California. Journal of Medical Entomology 54, 492-495.

Rulison, E.L., Kuczaj, I., Pang, G., Hickling, G.J., Tsao, J.I., Ginsberg, H.S., 2013. Flagging versus dragging as sampling methods for nymphal Ixodes scapularis (Acari: Ixodidae). J. Vector Ecol. 38, 163-167.

Rynkiewicz, E.C., Clay, K., 2014a. Tick community composition in Midwestern US habitats in relation to sampling method and environmental conditions. Experimental and Applied Acarology 64, 109-119.

Rynkiewicz, E.C., Clay, K., 2014b. Tick community composition in Midwestern US habitats in relation to sampling method and environmental conditions. Exp Appl Acarol 64, 109-119.

Savage, H.M., Burkhalter, K.L., Godsey, M.S., Panella, N.A., Ashley, D.C., Nicholson, W.L., Lambert, A.J., 2017. Bourbon Virus in Field-Collected Ticks, Missouri, USA. Emerging Infectious Diseases 23, 2017-2022.

Savage, H.M., Godsey, M.S., Panella, N.A., Burkhalter, K.L., Ashley, D.C., Lash, R.R., Ramsay, B., Patterson, T., Nicholson, W.L., 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, 607-612.

Savage, H.M., Godsey, M.S., Panella, N.A., Burkhalter, K.L., Manford, J., Trevino-Garrison, I.C., Straily, A., Wilson, S., Bowen, J., Raghavan, R.K., 2018a. Surveillance for Tick-Borne Viruses Near the Location of a Fatal Human Case of Bourbon Virus (Family Orthomyxoviridae: Genus Thogotovirus) in Eastern Kansas, 2015. Journal of Medical Entomology 55, 701-705.

Savage, H.M., Godsey, M.S., Tatman, J., Burkhalter, K.L., Hamm, A., Panella, N.A., Ghosh, A., Raghavan, R.K., 2018b. Surveillance for Heartland and Bourbon Viruses in Eastern Kansas, June 2016. Journal of Medical Entomology 55, 1613-1616.

Scharf, W.C., 2004. Immature ticks on birds: Temporal abundance and reinfestation. Northeastern Naturalist 11, 143-150.

Scharf, W.C., Walker, E.D., 2002. New host records for Ixodes muris (Acarina : Ixodidae) from Michigan birds. Great Lakes Entomologist 35, 113-114.

Schulze, T.L., Jordan, R.A., Dolan, M.C., 2011. Experimental Use of Two Standard Tick Collection Methods to Evaluate the Relative Effectiveness of Several Plant-Derived and Synthetic Repellents Against Ixodes scapularis and Amblyomma americanum (Acari: Ixodidae). Journal of Economic Entomology 104, 2062-2067.

Schulze, T.L., Jordan, R.A., Hung, R.W., 1997. Biases associated with several sampling methods used to estimate abundance of Ixodes scapularis and Amblyomma americanum (Acari : Ixodidae). Journal of Medical Entomology 34, 615-623.

Scott, J.D., Durden, L.A., 2014. Songbird-transported tick Ixodes minor (Ixodida: Ixodidae) discovered in Canada. The Canadian Entomologist 147, 46-50.

Scott, J.D., Durden, L.A., 2015. New records of the Lyme disease bacterium in ticks collected from songbirds in central and eastern Canada. International Journal of Acarology 41, 241-249.

Scott, J.D., Fernando, K., Banerjee, S.N., Durden, L.A., Byrne, S.K., Banerjee, M., Mann, R.B., Morshed, M.G., 2001. Birds disperse ixodid (Acari : Ixodidae) and Borrelia burgdorferi-infected ticks in Canada. Journal of Medical Entomology 38, 493-500.

Scott, J.D., Foley, J.E., Young, M.R., Durden, L.A., 2017. First report of a blacklegged tick, Ixodes scapularis Say (Acari: Ixodidae), parasitizing a raptor in Canada. Systematic and Applied Acarology 22.

Smith, K.A., Oesterle, P.T., Jardine, C.M., Dibernardo, A., Huynh, C., Lindsay, R., Pearl, D.L., Nemeth, N.M., 2019. Tick infestations of wildlife and companion animals in Ontario, Canada, with detection of human pathogens in Ixodes scapularis ticks. Ticks Tick-Borne Dis. 10, 72-76.

Solberg, V.B., Neidhardt, K., Sardelis, M.R., Hildebrandt, C., Hoffmann, F.J., Boobar, L.R., 1992a. QUANTITATIVE-EVALUATION OF SAMPLING METHODS FOR IXODES-DAMMINI AND AMBLYOMMA-AMERICANUM (ACARI, IXODIDAE). Journal of Medical Entomology 29, 451-456.

Solberg, V.B., Neidhardt, K., Sardelis, M.R., Hildebrandt, C., Hoffmann, F.J., Boobar, L.R., 1992b. Quantitative Evaluation of Sampling Methods for Ixodes dammini and Amblyomma americanum (Acari: Ixodidae). Journal of Medical Entomology 29, 451-456.

Sonenshine, D.E., 2018. Range Expansion of Tick Disease Vectors in North America: Implications for Spread of Tick-Borne Disease. Int. J. Environ. Res. Public Health 15, 9.

Soucy, J.R., Slatculescu, A.M., Nyiraneza, C., Ogden, N.H., Leighton, P.A., Kerr, J.T., Kulkarni, M.A., 2018. High-Resolution Ecological Niche Modeling of Ixodes scapularis Ticks Based on Passive Surveillance Data at the Northern Frontier of Lyme Disease Emergence in North America. Vector Borne Zoonotic Dis 18, 235-242.

Spencer, R.R., 1929. Rocky Mountain Spotted Fever. The Journal of Infectious Diseases 44, 257-276.

Springer, Y.P., Hoekman, D., Johnson, P.T.J., Duffy, P.A., Hufft, R.A., Barnett, D.T., Allan, B.F., Amman, B.R., Barker, C.M., Barrera, R., Beard, C.B., Beati, L., Begon, M., Blackmore, M.S., Bradshaw, W.E., Brisson, D., Calisher, C.H., Childs, J.E., Diuk-Wasser, M.A., Douglass, R.J., Eisen, R.J., Foley, D.H., Foley, J.E., Gaff, H.D., Gardner, S.L., Ginsberg, H.S., Glass, G.E., Hamer, S.A., Hayden, M.H., Hjelle, B., Holzapfel, C.M., Juliano, S.A., Kramer, L.D., Kuenzi, A.J., LaDeau, S.L., Livdahl, T.P., Mills, J.N., Moore, C.G., Morand, S., Nasci, R.S., Ogden, N.H., Ostfeld, R.S., Parmenter, R.R., Piesman, J., Reisen, W.K., Savage, H.M., Sonenshine, D.E., Swei, A., Yabsley, M.J., 2016. Tick-, mosquito-, and rodent-borne parasite sampling designs for the National Ecological Observatory Network. Ecosphere 7, 40.

Springer, Y.P., Taylor, J.R., Travers, P.D., 2015. Variation in Baiting Intensity Among CO2-Baited Traps Used to Collect Hematophagous Arthropods. J Insect Sci. 15, 10.

Stromdahl, E.Y., Hickling, G.J., 2012. Beyond Lyme: aetiology of tick-borne human diseases with emphasis on the south-eastern United States. Zoonoses Public Health 59 Suppl 2, 48-64.

Strong-Klefenz, J.E., Gaskill, C.L., 2008. A possible canine tick-bite reaction to Ixodes muris. Canadian Veterinary Journal-Revue Veterinaire Canadienne 49, 280-282.

Sumner, J.W., Durden, L.A., Goddard, J., Stromdahl, E.Y., Clark, K.L., Reeves, W.K., Paddock, C.D., 2007. Gulf coast ticks (Amblyomma maculatum) and Rickettsia parkeri, United States. Emerging infectious diseases 13, 751.

Tack, W., Madder, M., De Frenne, P., Vanhellemont, M., Gruwez, R., Verheyen, K., 2011. The effects of sampling method and vegetation type on the estimated abundance of Ixodes ricinus ticks in forests. Exp Appl Acarol 54, 285-292.

Teel, P.D., Hopkins, S.W., Donahue, W.A., Strey, O.F., 1998. Population dynamics of immature Amblyomma maculatum (Acari : Ixodidae) and other ectoparasites on meadowlarks and northern bobwhite quail resident to the coastal prairie of Texas. Journal of Medical Entomology 35, 483-488.

Teel, P.D., Ketchum, H.R., Mock, D.E., Wright, R.E., Strey, O.F., 2010. The Gulf Coast Tick: A Review of the Life History, Ecology, Distribution, and Emergence as an Arthropod of Medical and Veterinary Importance, Vol 47. SPIE, 16 p.

Theuret, D.P., Trout Fryxell, R.T., 2018. Beefing Up Biosecurity: Survey of Ticks (Acari: Ixodidae) Currently Threatening the Tennessee Beef Cattle Industry, and a Proposed Monitoring Strategy for Invasive Ticks. Journal of Medical Entomology, tjy131-tjy131.

Tietjen, M., Esteve-Gassent, M.D., Medina, R.F., 2019. Searching for the Immature Stages of Ixodes scapularis (Acari: Ixodidae) in Leaf Litter and Soil in Texas. Journal of Medical Entomology 56, 35-39.

Trout Fryxell, R.T., Moore, J.E., Collins, M.D., Kwon, Y., Jean-Philippe, S.R., Schaeffer, S.M., Odoi, A., Kennedy, M., Houston, A.E., 2015. Habitat and Vegetation Variables Are Not Enough When Predicting Tick Populations in the Southeastern United States. PLoS One 10, e0144092.

Trout, R., Steelman, C., Szalanski, A., Loftin, K., 2010. Establishment of amblyomma maculatum (Gulf Coast tick) in Arkansas, USA. The Florida Entomologist 93, 120-122.

Tulloch, J.S.P., McGinley, L., Sanchez-Vizcaino, F., Medlock, J.M., Radford, A.D., 2017. The passive surveillance of ticks using companion animal electronic health records. Epidemiology and Infection 145, 2020-2029.

Vasconcelos, P.F.C., Calisher, C.H., 2016. Emergence of Human Arboviral Diseases in the Americas, 2000-2016. Vector-Borne and Zoonotic Diseases 16, 295-301.

Wagner, N., Jappe, U., Podda, M., Hartmann, K., 2012. Anaphylaxis to red meat: galactose-alpha-1,3-galactose (alpha-Gal) - an interesting allergen. Allergologie 35, 559-562.

Washburn, A.M., Tuohy, J.H., 1949. The changing picture of tularemia transmission in Arkansas; a study of 704 case histories. Southern medical journal 42, 60-62.

Wilson, J.G., Sauer, J.R., Hair, J.A., Kinzer, D.R., 1972. CHEMO-ATTRACTION IN LONE STAR TICK (ACARINA-IXODIDAE) .1. RESPONSE OF DIFFERENT DEVELOPMENTAL STAGES TO CARBON-DIOXIDE ADMINISTERED VIA TRAPS. Journal of Medical Entomology 9, 245-&.

Wormser, G.P., Pritt, B., 2015. Update and Commentary on Four Emerging Tick-Borne Infections Ehrlichia muris-like Agent, Borrelia miyamotoi, Deer Tick Virus, Heartland Virus, and Whether Ticks Play a Role in Transmission of Bartonella henselae. Infect. Dis. Clin. North Am. 29, 371-+.

Wormser, G.P., Shapiro, E.D., Fish, D., 2019. Borrelia miyamotoi: An Emerging Tick-Borne Pathogen. American Journal of Medicine 132, 136-137.

Wright, C.L., Gaff, H.D., Sonenshine, D.E., Hynes, W.L., 2015a. Experimental vertical transmission of Rickettsia parkeri in the Gulf Coast tick, Amblyomma maculatum. Ticks Tick Borne Dis 6, 568-573.

Wright, C.L., Sonenshine, D.E., Gaff, H.D., Hynes, W.L., 2015b. Rickettsia parkeri Transmission to Amblyomma americanum by Cofeeding with Amblyomma maculatum (Acari: Ixodidae) and Potential for Spillover. J Med Entomol 52, 1090-1095.

Yabsley, M.J., Nims, T.N., Savage, M.Y., Durden, L.A., 2009. TICKS AND TICK-BORNE PATHOGENS AND PUTATIVE SYMBIONTS OF BLACK BEARS (URSUS AMERICANUS FLORIDANUS) FROM GEORGIA AND FLORIDA. Journal of Parasitology 95, 1125-1128.

Yoder, J.A., Pekins, P.J., Lorenz, A.L., Nelson, B.W., 2017. Larval behaviour of the winter tick, Dermacentor albipictus (Acari:Ixodidae): evaluation of CO2 (dry ice), and short- and long-range attractants by bioassay. International Journal of Acarology 43, 187-193.

Yunik, M.E.M., Galloway, T.D., Lindsay, L.R., 2015. Assessment of Prevalence and Distribution of Spotted Fever Group Rickettsiae in Manitoba, Canada, in the American Dog Tick, Dermacentor variabilis (Acari: Ixodidae). Vector-Borne and Zoonotic Diseases 15, 103-108.

CHAPTER 3:
COMPARISON OF DIFFERENT SURVEILLANCE METHODS FOR MODELING DISPERSAL OF TICKS[1]

[1]Koser, T, S Wyckoff, A James, M Wehtje, J Corn, and MJ Yabsley. To be submitted to *Ticks and Tick-borne Diseases*

Abstract

Ticks are important vectors for several pathogens and active surveillance through tick collection is an effective way to assess risk to tick-borne disease. In this study we compared the efficiencies of three tick collection methods: tick drags, CO_2-baited drags, and on-host extractions. On-host tick extractions detected seven tick species compared to four species with traditional tick drags and four species with CO_2-baited drags. For the two tick species collected using all methods, on-host extractions collected more *Ixodes scapularis* overall whereas CO_2-baited tick drags collected more *Amblyomma americanum*. On-host tick extractions involved greater time and monetary investment for effective deployment, but focusing on specific wildlife groups makes this technique much more efficient. Pathogen prevalence in environmentally-collected ticks was similar to previous research. Comparing these active tick surveillance methods provides important information for researchers to inform the employment effective collection methods given their interests and resource limitations.

INDEX WORDS: ticks, collection, pathogens, *Amblyomma americanum*, *Ixodes scapularis*

Introduction

Ticks are important vectors for human and companion animal pathogens and can be serious pests for livestock and wild animal populations. They transmit the highest diversity of pathogens of any arthropod vector and are responsible for 95% of all nationally notifiable human vector-borne disease cases reported to the Centers for Disease Control and Prevention (CDC) every year (Eisen et al., 2017). Ticks can also transmit pathogens or otherwise cause damage to economically important livestock populations, like the US$105 billion cattle industry (Theuret and Trout Fryxell, 2018), as well as to susceptible wildlife populations, such as *Alces alces* (Addison, 2007). There are several tick species in the Southeast that are important because they contribute to human and animal disease cases, including: *Amblyomma americanum* (the Lone Star tick), *Amblyomma maculatum* (the Gulf Coast tick), *Dermacentor variabilis* (the American Dog tick), and *Ixodes scapularis* (the Blacklegged tick). These tick species are capable of transmitting a wide array of different pathogens such as *Anaplasma phagocytophilum* (the causative agent for anaplasmosis), *Borrelia burgdorferi sensu stricto* (the causative agent for Lyme borreliosis), and multiple *Ehrlichia* spp. and *Rickettsia* spp. (the causative agents for ehrlichiosis and rickettsiosis) (Stromdahl and Hickling, 2012). The effective deployment of public health and animal management interventions to address tick-borne diseases relies upon active surveillance for ticks to represent risk for human and animal encounters with specific tick species and their associated pathogens.

Active surveillance for ticks can be divided into two broad categories: off-host or questing-tick collection (i.e. dragging, flagging, CO^2 traps) and on-host collection (i.e. wildlife sampling, domestic animal sampling, sentinel animal deployment). Different information relevant to tick research can be obtained from these methods and they are associated with their

own logistic and economic costs. For example, collection by dragging and flagging is relatively simple and cost-effective, yields information on tick abundance and density, and can be used to understand epidemiologically important relationships like tick habitat associations and phenology (Estrada-Pena et al., 2013). Ticks collected via these methods can also be tested for various pathogens, whereas ticks from hosts could carry pathogens from their hosts that they could not maintain into their next stage, making pathogen screening for estimating risk to humans problematic. Sampling wildlife populations and even livestock or domestic animals can be prohibitively expensive, time consuming, or procedurally difficult for researchers, but these methods provide critical information about tick community dynamics. Some tick species, such as *Ixodes texanus*, or certain stages of tick species, such as *Ixodes scapularis* nymphs in the Southeast, have close associations with their hosts and are nearly exclusively collected from their wildlife hosts (Ouellette et al., 1997; Tietjen et al., 2019). Even though tick species like *Ixodes texanus* may not directly transmit pathogens to humans, they can still play a crucial role as enzootic vectors, maintaining pathogens in wildlife communities, making their detection crucial in informing regionally-specific aspects of a pathogen's enzootic cycle (Ouellette et al., 1997). Additionally, tick-host associations can differ for a single tick species across its range, as is the case for the Northern and Southern Clades of *Ixodes scapularis*, affecting tick questing behavior and pathogen prevalence, thus highlighting the importance of regional sampling of widespread generalist tick species in assessing local risk to ticks and tick-borne pathogens (McCoy et al., 2013).

Relatively few studies compare tick collection methods across multiple tick species (Chong et al., 2013b; Dantas-Torres et al., 2013; Ginsberg and Ewing, 1989; Li and Dunley, 1998; Mays et al., 2016a; Ramos Vdo et al., 2014; Rynkiewicz and Clay, 2014b; Schulze et al.,

1997; Solberg et al., 1992b; Terassini et al., 2010). Few of these studies sampled across multiple seasons and in different regions or even different habitats, as would be necessary for sampling entire tick communities (Estrada-Pena et al., 2013), and only one has done this in the Southeast (Mays et al., 2016a). Only one study in the United States has compared the tick species richness and abundance collected from off-host and on-host sampling methods (Rynkiewicz and Clay, 2014b), but there has not been a study comparing these methods in the Southeast or by sampling a broad diversity of tick hosts. The purpose of this study is to compare the number of tick species and respective abundances collected by three tick collection methods: tick dragging, CO^2-baited tick dragging, and on-host tick extraction. Methods were also compared by the amount of time and resources invested into their employment. We employed each collection method in two physiographic regions (the Piedmont and the Lower Coastal Plain), three habitats in each region, with three replicate sites sampled for each habitat type. We also sampled these sites seasonally from November 2017 – November 2018. Finally, ticks collected via the dragging and CO^2-baited dragging methods were screened for six different pathogens important to human and animal health.

Materials and Methods

Study Site

Each collection method was deployed in three different habitat types within two different physiographic regions in Georgia. In the Piedmont physiographic region we sampled the habitat types: mixed pine-hardwood forest, loblolly (*Pinus taeda*) pine stands, and bottomland hardwood forest. In the Lower Coastal Plain we sampled maritime forest, longleaf pine (*Pinus palustris*) stands, and bottomland hardwood forest habitats. The Piedmont sites were located in Oconee National Forest while the Lower Coastal Plain sites were located in Richmond Hill WMA. Three replicate sites for each habitat were chosen based on previous research (Pfaff et al., Unpublished research), yielding a total of 18 sites. One loblolly pine stand site in the Piedmont region was moved due to logging in Spring 2018 and one mixed pine-hardwood forest site in the Piedmont region was moved in Summer 2018 due to prescribed fire. Other than these two sites for two habitat types in the Piedmont, each site was sampled seasonally between 2018 and 2019: winter collections occurred during January and February, spring during April and May, summer during July, and fall during October and November.

Collection Methods

Ticks were collected using three different collection methods: tick dragging, CO^2-baited tick dragging, and on-host wildlife tick extraction. For each method, a record of time and resource investments was detailed for later comparisons. Tick dragging involved dragging a 1 m^2 flannel cloth attached to a wooden dowel across uniform patches of vegetation at each site (Rulison et al., 2013). Tick drags were conducted across twenty parallel 25 meter transects (500 m^2 for each site) at each site and drag clothes were checked for ticks every 10 steps.

Tick collection via CO^2-baited tick dragging was similar to tick dragging, with an added stimulus of dry ice. This type of collection method using two common tick methods: physical stimulus with a tick drag and a chemical stimulant with carbon dioxide, has been used previously (Gherman et al., 2012; Mays et al., 2016a), but this specific technique is novel. At each site over a 500 m^2 transect area, approximately 3 pounds of dry ice (frozen carbon dioxide) was chunked into pellets and scattered across the area. Tick draggers waited 30 minutes for the carbon dioxide to sublimate then conducted a tick drag as normal. CO^2-baited tick drags were conducted in transect areas that were separate from traditional drag transects yet still across similar vegetation. These areas were also sampled after using the traditional tick drag to mitigate the effects of attracting ticks in the area.

On-host tick extraction focused on three groups of important tick hosts: mesomammals (i.e. *Procyon lotor, Didelphis virginiana*), rodents (i.e. *Peromyscus gossypinus, Sigmodon hispidus, Neotoma floridana*), and passerine birds (i.e. *Cardinalis cardinalis, Spizella passerina, Melospiza melodia*). Mesomammals were trapped using forty 36 x 10 x 10 inch metal cage traps (Tomahawk Live Trap ©), rodents with thirty 6.5 x 2 x 2.5 inch and twenty 15 x 5 x 5 inch Sherman-style metal box traps (Tomahawk Live Trap ©), and passerine birds were captured using two 1.2 x 2.6 meter and one 1.2 x 5.2 meter mist net (Nixalite ©). Traps were deployed at locations where animal sign was visible (trails, markings, waste) across three sites for 4 days then rotated to the next three sites until all 9 sites per physiographic region were sampled. Mist nets were erected for three hours for three mornings at each site then rotated to the next site. All wildlife hosts were identified to species, age class, and sex if possible and inspected for ticks. We either manually restrained or chemically immobilized the animals if they could not be safely handled. All animal handling was approved by AUP IUCUC UGA (A2018 02-010-Y1-A1).

Collected ticks were stored in 70% ethanol and identified under a dissecting microscope using dichotomous keys (Keirans and Clifford, 1978; Keirans and Durden, 1998; Keirans and Litwak, 1989). Questionable larval ticks or ticks collected from wildlife with identifying features removed were identified to life stage and genus and identified using molecular methods.

Ten randomly selected points within drag transect areas were used for vegetation surveys. Canopy closure was measured with a spherical densiometer, shrub cover was measured using a line intercept method, and litter depth was estimated with a pin flag randomly dropped within a 1 m^2 frame at each point (Lemmon 1957). We also collected microclimate measurements at three randomly selected points in each transect area using a Kestrel Weather Meter (Loftopia LLC, Birmingham, MI). The microclimate measurements collected were temperature, relative humidity and wind speed at ground level and 1 m above ground.

Pathogen Screening

Adult ticks collected via the dragging and CO^2-baited dragging methods were bisected and DNA was extracted from half the tick using a commercial kit (Qiagen DNeasy Blood and Tissue kit, Valencia, CA) and the other half was stored in empty, labeled tubes in a -20º C freezer. We screened the extracted tick DNA for six pathogens using protocols outlined in Table 1.1. Each pathogen required a nested polymerase chain reaction (PCR) protocol. Each primary reaction (except for Barbour et al. (1996)) involved assembling 25 µL volumes consisting of 11µL of molecular grade biological water (MGBW), 2.5 µL of $MgCl_2$, 5 µL of GoTaq Flexi Clear Buffer (Promega, Madison, WI), 0.25 µL of DNTPs (20 mM initial concentration), 0.5 µL of each primer (50 µM initial concentration), 0.25 µL of GoTaq Flexi (Promega, Madison, WI), and 5 µL of extracted tick DNA. The *Borrelia fla* primary procedure involved using 10 µL of extracted DNA and 6 µL of MGBW (Barbour et al., 1996). The secondary reactions were

similar, also reaching 25 µL in total volume, but 11 µL of MGBW was used and 1 µL of primary PCR product. Negative controls of MGBW in place of extracted DNA or PCR product were included at the tick DNA extraction, primary PCR, and secondary PCR steps and a positive control for a given pathogen was included in the primary PCR step for each batch of ticks. Batches typically included 20 ticks of one species being tested for the same pathogens. Each step was conducted in separate laboratory areas in dedicated hoods equipped with UV lights for decontamination between screening batches.

Secondary PCR products were separated using gel electrophoresis on a 2.5% agarose gel stained with GelRed (Biotium, Fremont, CA). After electrophoresis was completed, gels were visualized using UV light from an AlphaImager (ProteinSimple, San Jose, CA). All samples positive for *Borrelia* or Panola Mountain *Ehrlichia* and subsets of positive *Rickettsia* samples were extracted with a gel extraction kit (Qiagen) for bi-directional sequencing at Georgia Genomics Facility (Athens, GA). Sequences were then compared to available sequences on GenBank.

Table 3.1 Established PCR protocols for isolating gene targets from selected pathogens.

Pathogen	Tick Species Screened	PCR gene target	Target size (bp)	Primers (Primary and Secondary)	References
Rickettsia spp.	*A. americanum* *A. maculatum* *D. variabilis*	17kDa	434	17k5/17k3 and 17lD1/17kD2	(Labruna et al., 2004)
Ehrlichia chaffeensis	*A. americanum* *A. maculatum* *D. variabilis*	16S rRNA	389	ECC/ECB and HE1/HE3	(Dawson et al., 1996)
Ehrlichia ewingii	*A. americanum* *A. maculatum* *D. variabilis*	16S rRNA	400	ECC/ECB and EE72/HE3	(Anderson et al., 1992a)
Panola Mountain *Ehrlichia*	*A. americanum*	gltA	405	CS-185F/CS-777R (primary) and CS-214F/CS-619R (secondary)	(Loftis et al., 2008)

Anaplasma phagocytophilum	*Ixodes scapularis* *Ixodes affinis*	16S rRNA	410	ECC/ECB (primary) and GE9F/GAU1R (secondary)	(Little et al., 1997)
Borrelia spp.	*Ixodes scapularis* *Ixodes affinis*	flaB	330	FLALL/FLARL (primary) and FLALS/FLARS (secondary)	(Barbour et al., 1996)

Statistical analysis

All analyses were completed in RStudio 1.1.463 (2019). Zero-Inflated Poisson Generalized Linear Mixed Models (ZIP GLMM) were used to compare collection methods across both physiographic regions, all habitat types (nested within physiographic regions), and across all four seasons (repeated measures) with site included as a random factor (Bolker et al., 2012). *Amblyomma americanum* and *Ixodes scapularis* were the only tick species collected using all three collection methods, so ZIP GLMMs analyzing the efficiency of collection methods across all of the previously described factors were also conducted individually on these two species. We compared AIC values and used likelihood ratio tests to compare variable predicting power to a null model to determine the best-fit model. Multiple comparisons for all three (all ticks, *Amblyomma americanum*, *Ixodes scapularis*) ZIP GLMMs by factor level were conducted by separating means with pairwise comparisons and Tukey-Kramer adjustment.

Results

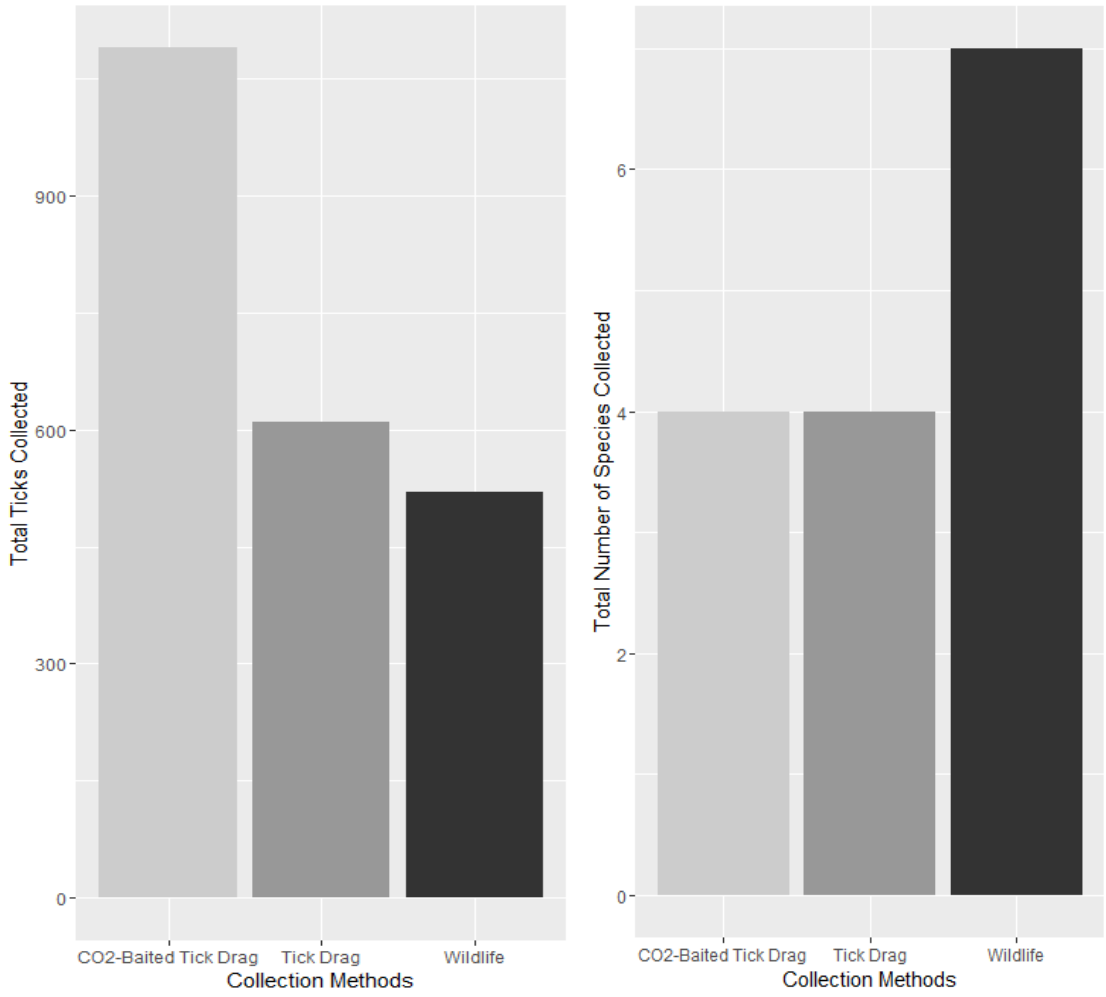
In total we collected 2,218 ticks of 8 species using 3 collection methods across 18 sites over the course of 4 seasons. Of these, 609 ticks of four species (558 *Amblyomma americanum*, 44 *Ixodes scapularis*, *3 Amblyomma maculatum*, and 4 *Ixodes affinis*) were collected via tick drags. For CO^2-baited drags, 1089 ticks of four species (1029 *A. americanum*, 57 *I. scapularis*, 1 *Ixodes affinis*, and 2 *Dermacentor variabilis*) were collected. *A. americanum* larval clusters were recorded as a single tick for this study. Ticks collected off wildlife hosts included 520

individuals of seven species (125 *A. americanum*, 152 *Ixodes scapularis*, 121 *D. variabilis*, 45 *Ixodes texanus*, 73 *A. maculatum*, 3 *Haemaphysalis leporispalustris*, and 1 *Ixodes muris*).

The collection method, field season, and habitat factors included in the total ticks ZIP GLMM had significant effects on total ticks collected (all factors AIC = 1875.7, LR Test p<0001). Overall, collection method had a significant effect on the total number of ticks collected (z_{ATD} = -6.48, p < 0.0001, z_{TD} = -3.87, p<0.0001, $z_{wildlife}$ = 2.32, p<0.0001)('ATD' = 'Augmented tick drag' or 'CO^2-baited tick drag', 'TD' = Traditional ick drag, 'Wildlife' = on-host tick extractions), with the CO^2-baited tick drags collecting more ticks (Figure 3.1) than both the traditional tick drag and on-host tick extraction methods. The on-host tick collection method detected 7 species of tick total, including three species not collected in the environmental sampling methods (Figure 3.2). The three methods also varied in the number of ticks they collected by physiographic region (Figure 3.3), but not significantly (z = -0.822, p = 0.411). Tick collection method efficiency varied again over the 6 habitat types sampled ($z_{BLH(P)}$=4.33, p<0.0001, z_{MF}=0.74, p=0.46, z_{Pine}=-1.02, p=0.308, $z_{Pine(P)}$=-2.17, p=0.03, z_{UMH}=0.99, p=0.32, z_{BLH}=3.78, p<0.0001,) (Figure 1.4), with more ticks collected with CO^2-baited tick drags in both bottomland hardwood forest habitats and maritime forest habitat, but within the upland mixed forest habitat of the Piedmont region, on-host tick extraction collected significantly more ticks than all other collection methods (z value = -14.886, p < 0.0001). Total ticks collected across field seasons also varied significantly (z_{Fall} = 10.067, p <0.0001, z_{Winter} = 12.148, p <0.0001, z_{Spring} = 8.047, p <0.0001, z_{Summer} = 6.660, p <0.0001) (Figure 1.5), with CO^2-baited tick drags collecting significantly more ticks in the spring, summer, and winter seasons (z range : 3.866 - -3.854, p<0.0001) while on-host tick extraction collected more ticks in the fall (z=-3.854, p<0.0001).

Two species of tick were collected with all three collection methods: *Amblyomma americanum* and *Ixodes scapularis*. Overall, *A. americanum* was collected more often with CO^2-baited tick drags ($z=15.79$, $p<0.0001$), but this is especially true for the bottomland hardwood forest habitats ($z=4.88$, $p<0.0001$), upland mixed forest habitat ($z=-4.93$, $p<0.0001$), and maritime forest habitat ($z=-5.48$, $p<0.0001$) and in the spring field season ($z=-6.32$, $p<0.0001$). *Ixodes scapularis* was collected less uniformly with one collection method (p range : 0.113-0.490), but was collected in much higher numbers using the on-host wildlife extraction method specifically in the upland mixed forest habitat ($z=2.398$, $p=0.032$).

The wildlife group with both the highest infestation and prevalence rate was, by far, mesomammals, with an infestation prevalence rate of 47.26% and an infestation density of roughly 4 ticks per infested individual. Both *Procyon lotor* and *Didelphis virginiana* demonstrated similar tick species diversities, with 4 tick species found on each, and approximately half of the individuals sampled for these two species across all seasons had some tick burden (56.52 % and 44.13 %). Prevalence of infestation was much lower for the rodent and passerine wildlife groups (5.65 – 25% and 9.58%).



Figures 3.1 & 3.2:

Total number of ticks collected with three different collection methods (CO_2-baited tick drags, traditional tick drags, and wildlife tick extractions). Ticks were collected seasonally from 3 replicate sites for 3 different habitat types in two physiographic regions of Georgia.

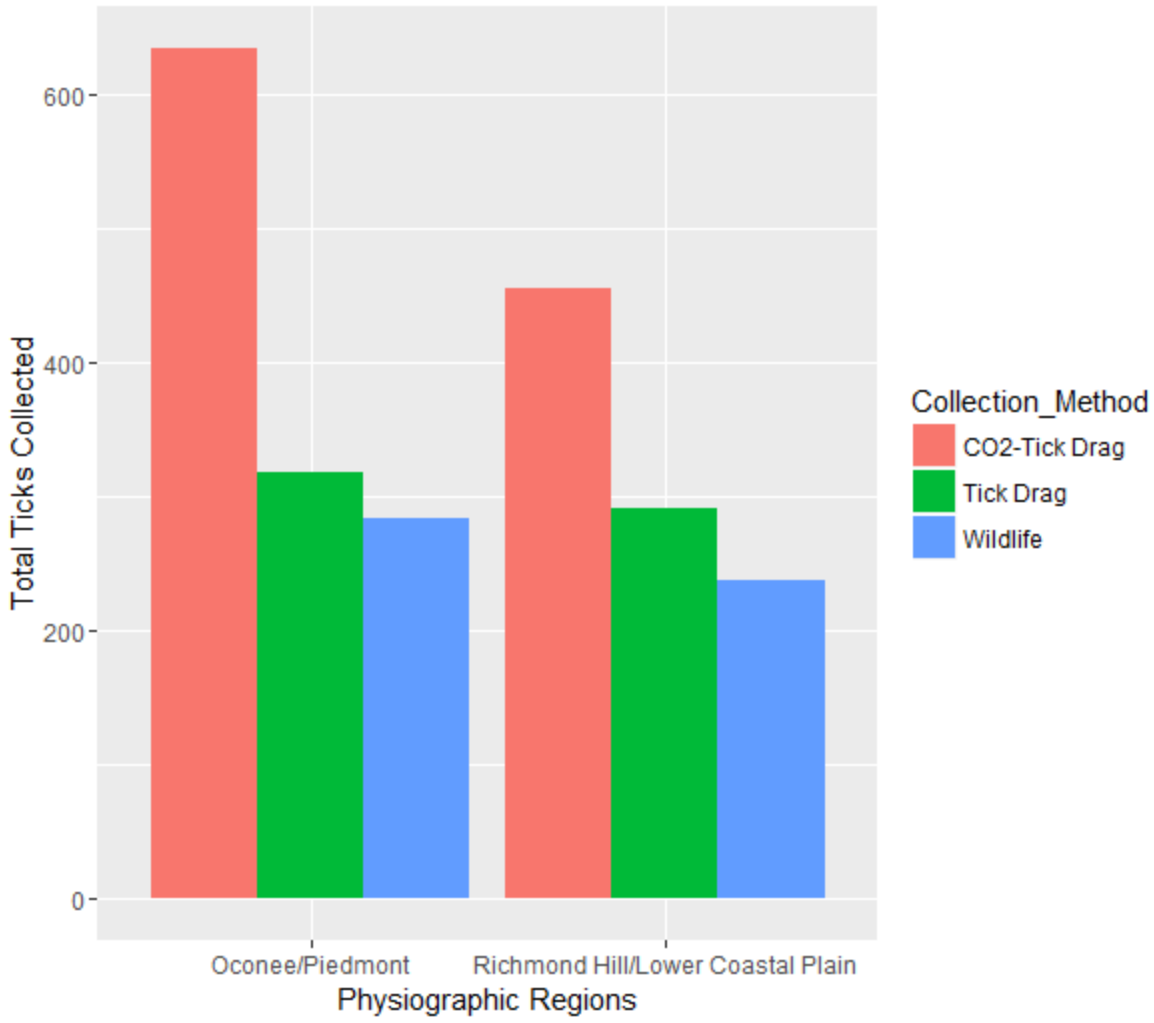


Figure 3.3:

Total number of ticks collected with three different collection methods (CO_2-baited tick drags, traditional tick drags, and wildlife tick extractions) in two physiographic regions of Georgia: the Piedmont and Lower Coastal Plain regions. Ticks were collected seasonally from 3 replicate sites for 3 different habitat types in each region.

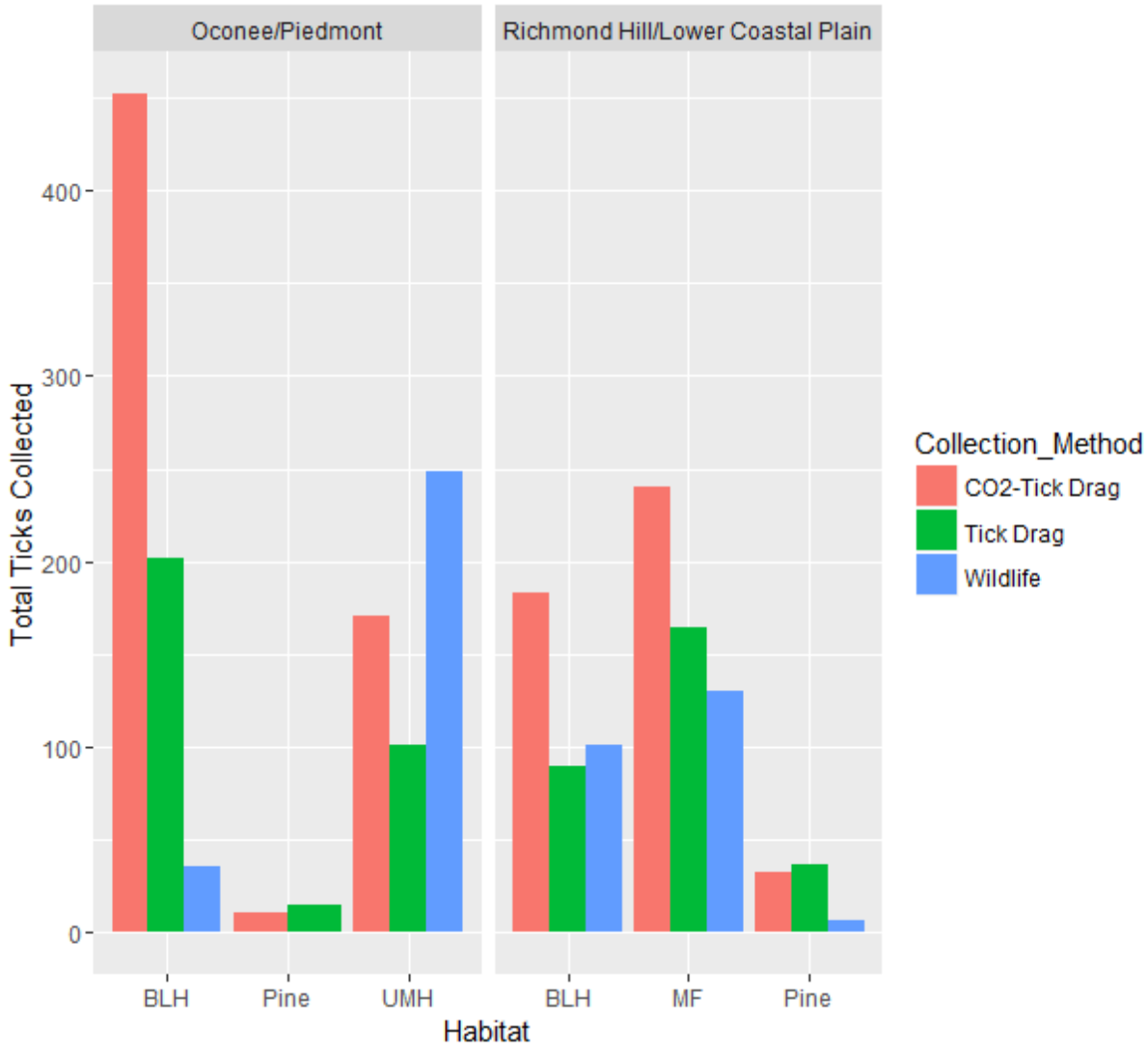


Figure 3.4:

Total number of ticks collected seasonally with three different collection methods (CO_2-baited tick drags, traditional tick drags, and wildlife tick extractions) in 3 habitat types and in two physiographic regions. Bottomland Hardwood Forest (BLH), Loblolly Pine Forest (Pine), and Upland Mixed Hardwood Forest (UMH) were sampled in the Piedmont region and Bottomland Hardwood Forest (BLH), Maritime Forest (MF), and Longleaf Pine Forest were sampled in the Lower Coastal Plain region.

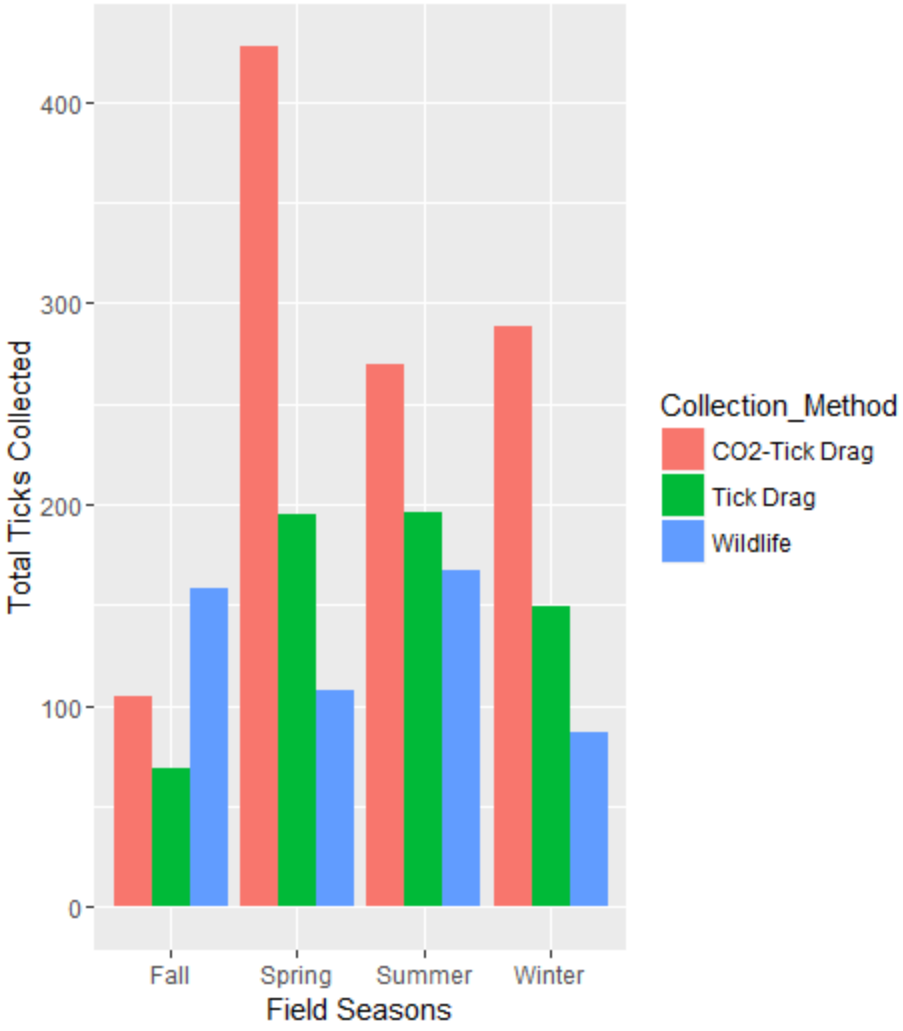


Figure 3.5:

Total number ticks collected with three different collection methods (CO_2-baited tick drags, traditional tick drags, and wildlife tick extractions) across four collection seasons spanning 2018-2019. Ticks were collected in 3 different habitat types in two physiographic regions of Georgia.

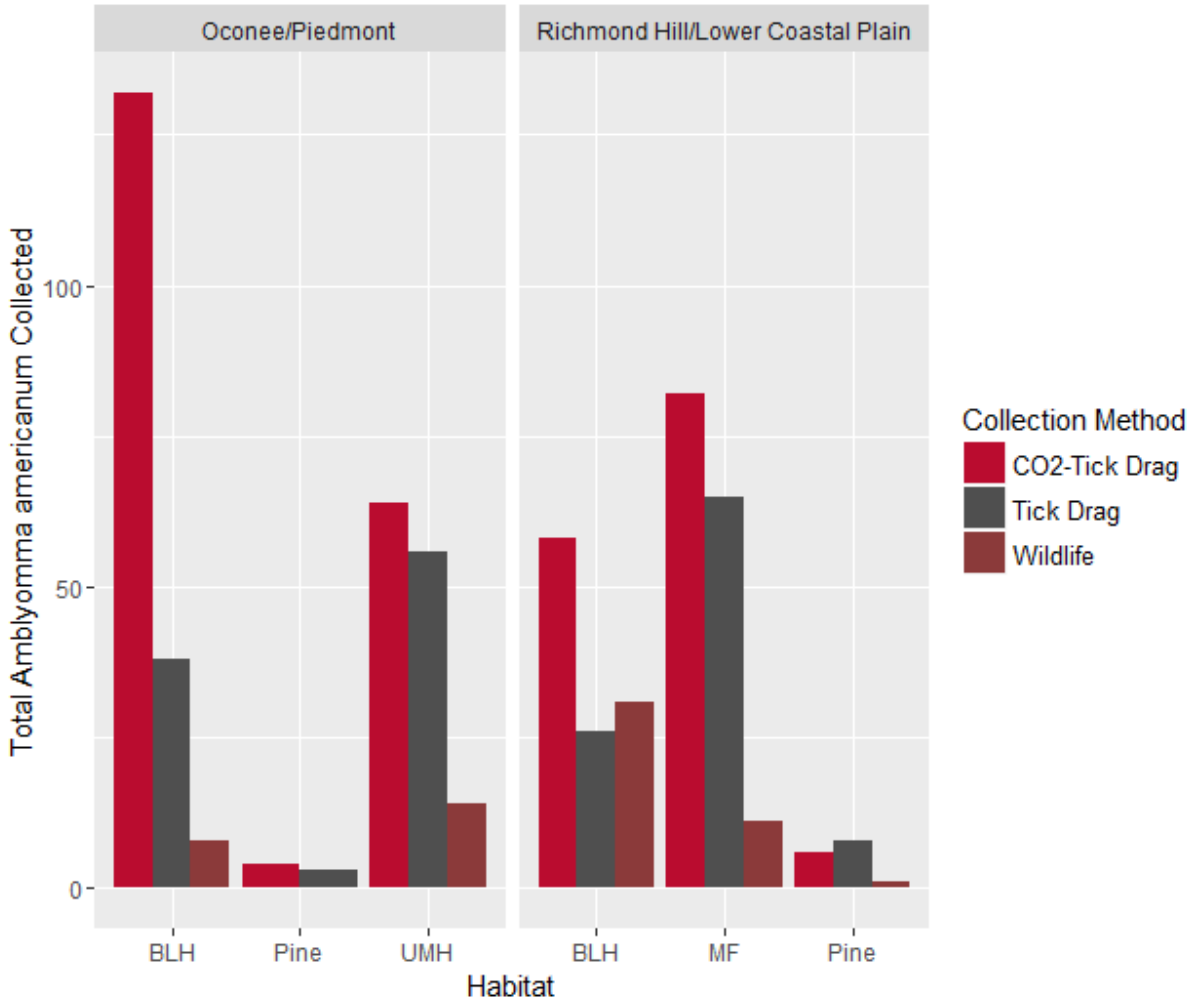


Figure 3.6:

Total number of *Amblyomma americanum* collected seasonally with three different collection methods (CO_2-baited tick drags, traditional tick drags, and wildlife tick extractions) in 3 habitat types and in two physiographic regions. Bottomland Hardwood Forest (BLH), Loblolly Pine Forest (Pine), and Upland Mixed Hardwood Forest (UMH) were sampled in the Piedmont region and Bottomland Hardwood Forest (BLH), Maritime Forest (MF), and Longleaf Pine Forest were sampled in the Lower Coastal Plain region.

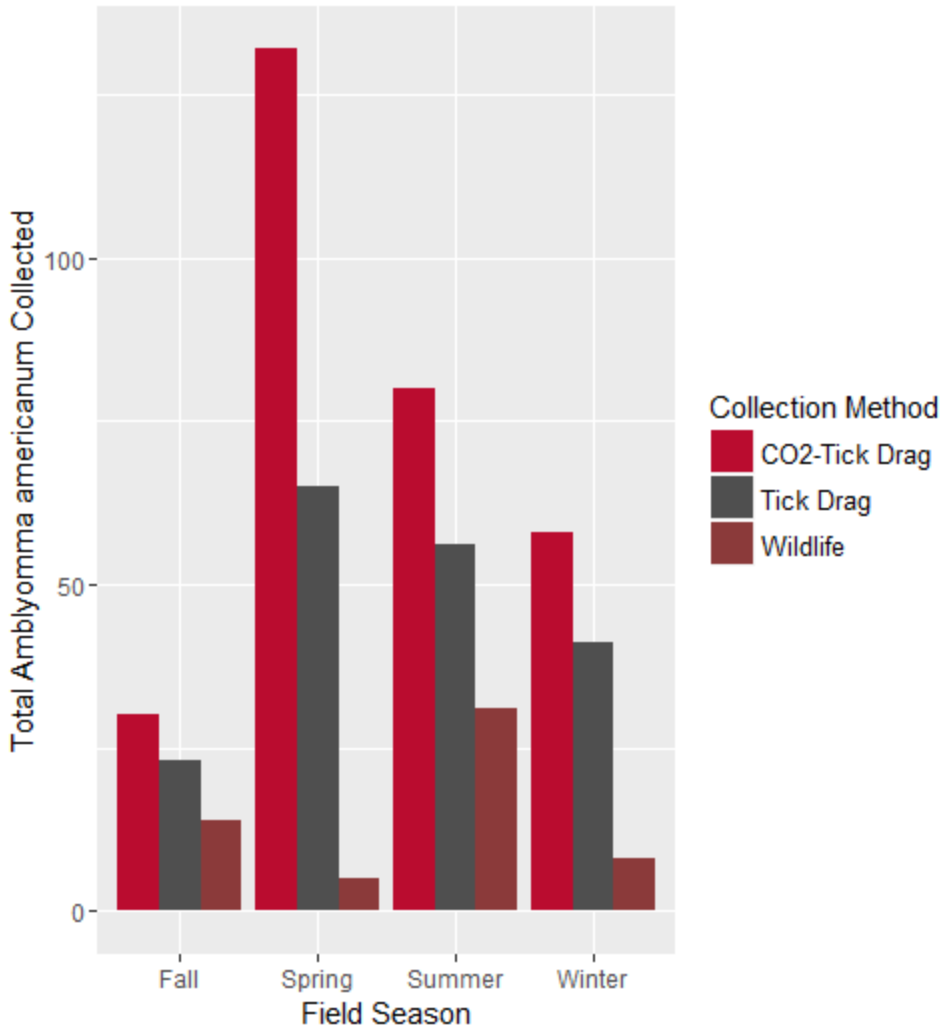


Figure 3.7:

Total number of *Amblyomma americanum* collected with three different collection methods (CO_2-baited tick drags, traditional tick drags, and wildlife tick extractions) across four collection seasons spanning 2018-2019. Ticks were collected in 3 different habitat types in two physiographic regions of Georgia.

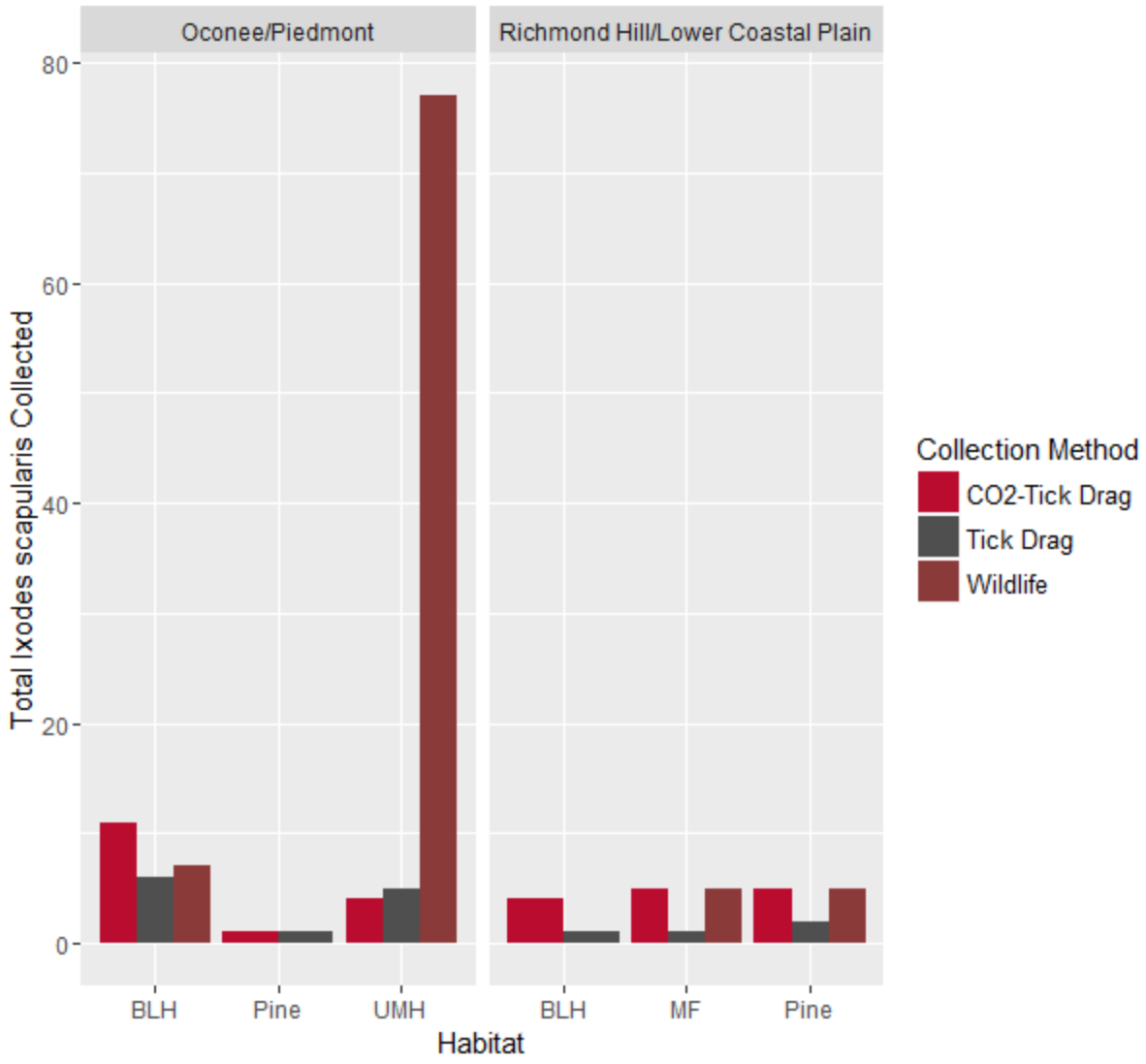


Figure 3.8:

Total number of *Ixodes scapularis* collected seasonally with three different collection methods (CO_2-baited tick drags, traditional tick drags, and wildlife tick extractions) in 3 habitat types and in two physiographic regions. Bottomland Hardwood Forest (BLH), Loblolly Pine Forest (Pine), and Upland Mixed Hardwood Forest (UMH) were sampled in the Piedmont region and Bottomland Hardwood Forest (BLH), Maritime Forest (MF), and Longleaf Pine Forest were sampled in the Lower Coastal Plain region.

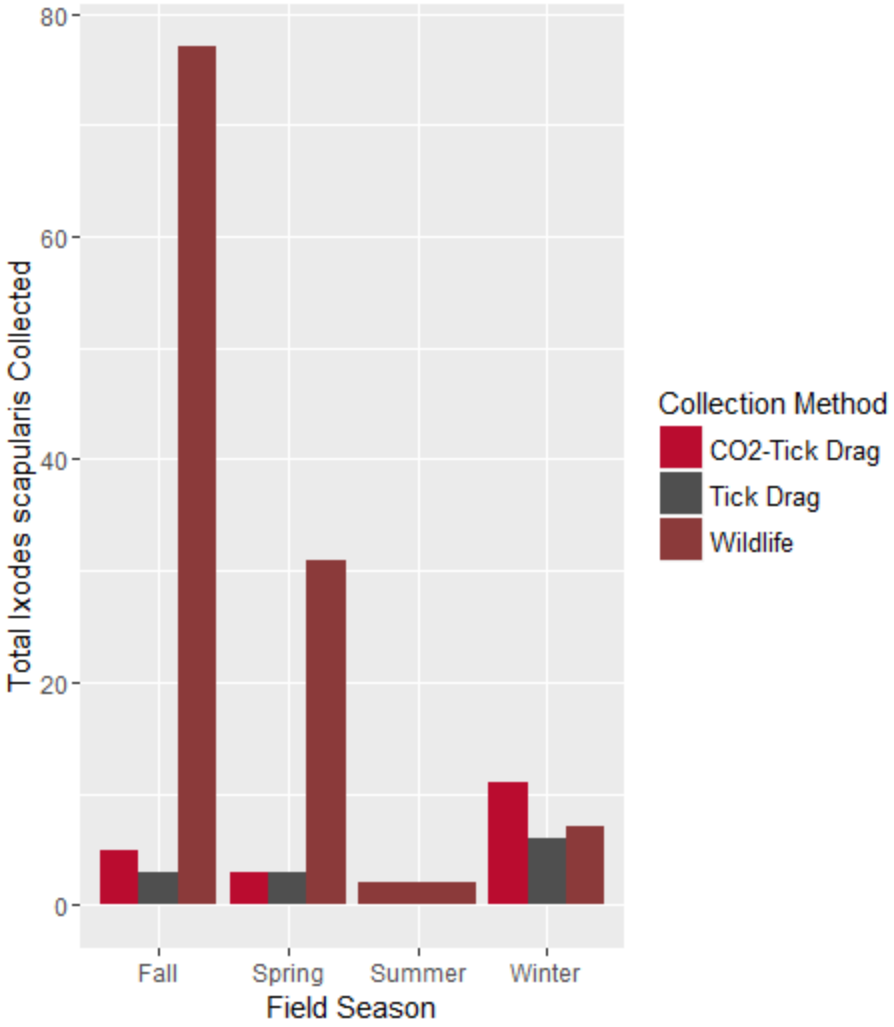


Figure 3.9:

Total number of *Ixodes scapularis* collected with three different collection methods (CO_2-baited tick drags, traditional tick drags, and wildlife tick extractions) across four collection seasons spanning 2018-2019. Ticks were collected in 3 different habitat types in two physiographic regions of Georgia.

Table 3.2:

Wildlife-host tick prevalence of infestation for all species collected (2nd column) and for individual tick species (columns 3-9) collected seasonally from 3 different habitats in two physiographic regions of Georgia, 2018-2019. A. amer=*Amblyomma americanum*, A. mac= *A. maculatum*, D. var= *D. variablis*, I. scap= *Ixodes scapularis*, I. tex= *I. texanus*, I. mur= *I. muris*, H. lepo= *H. leporispalustris*.

Hosts	No. infested /No sampled(%)	No. infested (Average number of ticks per infested host)						
		A. amer	*A. mac*	*D. var*	*I. scap*	*I. tex*	*I. mur*	*H. lepo*
Raccoon	26/46 (56.52)	12 (4)		9 (5)	6 (1)	7 (7)		
Opossum	79/179 (44.13)	8 (1)	1 (1)	30 (2)	44 (3)			
Grey Fox	3/3 (100)	1 (1)		1 (2)	3 (2)			
Eastern cottontail	3/6 (50)	1 (1)						2 (1)
Marsh rabbit	1/3 (33.33)	1 (1)						1(1)
Cotton rat	9/78 (11.54)		7 (7)		1 (1)			
Cotton mouse	6/129 (5.65)		5 (11)		1 (1)			
E. woodrat	2/8 (25)		2 (4)					
E. gray squirrel	1/12 (8.33)	1 (5)						
Passerine birds	23/240 (9.58)	9 (6)	5 (4)		9 (1)		1 (1)	1 (1)

Pathogen Prevalence

All adult ticks collected with the traditional tick drag and CO^2-baited tick drag were screened for associated pathogens except for a small number of representative individuals which were removed for establishing a voucher collection. Of the 318 *A. americanum* adults tested, 272 (85.53%) were positive for *Rickettsia* spp. and all sequenced PCR products were most

closely related to *Rickettsia amblyommatis*. All 3 *A. maculatum* and both *D. variabilis* tested for *Rickettsia* spp. were positive, with the *A. maculatum* samples closely resembling *Rickettsia tamurae* (2/3) and the other *A. maculatum* sample closely aligning with the Boutonneuse fever agent: *Rickettsia parkeri*. The *D. variabilis* samples most closely resembled *Rickettsia amblyommatis*. All *A. maculatum* and *D. variabilis* ticks tested for *E. chaffeensis*, *E. ewingii*, and Panola Mountain *Ehrlichia* were negative. For *A. americanum*, 17 out of 318 (5.35%) screened ticks were positive for *E. chaffeensis*, 18 out of 318 (5.66%) were positive for *E. ewingii*, and 12 out of 318 (3.77%) were positive for Panola Mountain *Ehrlichia* (Table 1.3).

Of the 99 *Ixodes scapularis* adults screened for *Borrelia* spp., only 2 (2.02%) came back as positive and their sequences most closely aligned with *Borrelia burgdorferi*. Out of the 3 adult *Ixodes affinis* screened for *Borrelia* spp., all 3 were positive with *Borrelia burgdorferi* as well. For *Anaplasma phagocytophilum*, 3 *Ixodes scapularis* adults were positive (3.03%) and none of the *Ixodes affinis* adults were positive (Table 1.3).

Table 3.3:

Pathogen prevalence for six tick-borne pathogens in 5 tick species collected seasonally with two different tick drag techniques in Georgia, 2018-2019.

	Pathogen					
Tick Species	*Rickettsia* spp.	*Ehrlichia chaffeensis*	*Ehrlichia ewingii*	Panola Mountain *Ehrlichia*	*Borrelia* spp.	*Anaplasma phagocytophilum*
A. americanum	272 / 318 (85.53%)	17 / 318 (5.35%)	18 / 318 (5.66%)	12 / 318 (3.77%)	N/A	N/A
A. maculatum	3 / 3 (100%)	0 / 3	0 / 3	0 / 3	N/A	N/A
D. variabilis	2 / 2 (100%)	0 / 2	0 / 2	0 / 2	N/A	N/A
Ixodes scapularis	N/A	N/A	N/A	N/A	2 / 99 (2.02%)	3 / 99 (3.03%)
Ixodes affinis	N/A	N/A	N/A	N/A	3 / 3 (100%)	0 / 3

Table 3.4:

Resource (monetary and time) investment for three different tick collection methods over the course of sampling at 18 sites repeatedly for 4 seasons. Included are resources such as traps, bait, ethanol and tubes but excluded are expenses such as gas, permit applications, chemical immobilization drugs and equipment.

Collection Method	Total Financial Investment	Per site Financial Investment	Time Investment (Nearest Hour)	Per site Time Investment (Nearest Hour)
Traditional Tick Drag	USD$ 97.37	USD$ 1.35	108 Hours	1.5 Hours
CO^2-Baited Tick Drag	USD$ 920.33	USD$ 12.78	144 Hours	2 Hours
On-host Tick Extraction	USD$ 5157.21	USD$ 71.63	1112 Hours	15 Hours
Mesomammal	USD$ 3819.00	USD$ 53.04	672 Hours	9 Hours
Rodents	USD$ 1237.50	USD$ 17.19	224 Hours	3 Hours
Passerine Birds	USD$ 101.21	USD$ 1.41	216 Hours	3 Hours

Resource Investment

Total time and monetary investment varied by collection method, with more money and time spent on on-host tick extractions ($ 5,157.21 and 1112 hours) than both the traditional and CO^2-baited tick drags. When examined further, the majority of the time (9/15 site hours) and money ($53.04 / $71.63 per site) invested in on-host tick extractions came from mesomammal trapping efforts specifically, with rodent and passerine investments more closely resembling those of the traditional and CO^2-baited tick drags.

Discussion

We evaluated the effectiveness of two environmental tick collection techniques, the traditional tick drag and the novel CO^2-baited tick drag, as well as on-host tick extraction and compared these methods based on the total number of ticks they collected, number of different species they collected, and the amount of resources invested into each method. The novel tick collection method collected a higher abundance of total ticks and was especially effective in the bottomland hardwood forest and maritime forest habitats (Figures 3.1 & 3.4), but the majority of these ticks were represented by one generalist species: *Amblyomma americanum* (Figure 3.6). As for collecting multiple tick species, the traditional tick drag and CO^2-baited tick drag methods detected 4 species of tick each (Figure 3.2), including the common tick species *Amblyomma americanum* and *Ixodes scapularis* as well as the less-frequently collected *Amblyomma maculatum*, *Dermacentor variabilis*, and *Ixodes affinis*. On-host tick extraction collected a similar overall tick abundance as the traditional tick drag technique, but detected a total of 7 tick species, including 3 (*Ixodes texanus*, *Ixodes muris*, and *Haemaphysalis leporispalustris*) tick species not collected with either environmental sampling technique. Only two tick species were collected using all three collection methods: *Amblyomma americanum* and *Ixodes scapularis*. The CO^2-baited tick drag method collected the greatest numbers of *Amblyomma americanum*, specifically in the bottomland hardwood, maritime, and upland mixed forest habitats (Figure 3.6) while the three collection methods generally faired similarly in collecting *Ixodes scapularis* across habitats except for the upland mixed forest habitat of the Piedmont region, where on-host tick extraction collected significantly higher *Ixodes scapularis* numbers.

As is evident in these results, the tick communities estimated in a specific habitat vary by which tick collection method is used. If specific tick species are targeted, then the use of an

efficient tick collection method for that species in the correct habitat type is recommended, but if multiple tick species are targeted then multiple tick collection methods are likely necessary to collect representative samples of each tick species in the community. Many studies have found that specific collection techniques employing a single stimulus, like a semiochemical stimulus such as carbon dioxide, will collect specific tick species or life stages, as with carbon dioxide and *A. americanum* adults and nymphs, thus yielding information on a single tick species and its pathogen prevalence but not necessarily representing the tick community as a whole (Ginsberg and Ewing, 1989). Research on tick-pathogen ecology, and specifically on the influences of tick species co-feeding and the presence of enzootic vectors on pathogen prevalence, has highlighted the importance of assessing not only the prevalence of tick-borne pathogens in human-biting vectors, but also the presence and pathogen prevalence of other tick species in these communities (Harrison et al., 2010; Ouellette et al., 1997; Wright et al., 2015b).

The employment of multiple tick collection methods does, however, come with the subsequent costs of deploying more equipment and spending more time at sample sites. One of the main reasons why the traditional tick drag is such a widely used tick collection method is because it is cheap, effective at collecting two tick species which are important human-biting vectors, and takes little time to employ (Table 3.3). Using another stimulus such as carbon dioxide in the form of dry ice is slightly more expensive and time-consuming than simply conducting a tick drag, but it does collect significantly higher numbers of *A. americanum*, which may prove important for pathogen surveillance studies. The on-host tick extraction method collects the greatest species richness of all the tick collection methods compared here, thus better representing the tick community than the other two collection methods, but this method required a much higher financial and time resource investment. This method may be the only technique,

however, to collect some important and infrequently environmentally detected species such as *Ixodes texanus, Ixodes muris,* and *Haemaphysalis leporispalustris*. On-host tick extraction also collected greater numbers of *D. variabilis* and *A. maculatum,* found on the mesomammal group and the rodent or bird groups respectively, than the other collection methods, making the targeting of on-host tick extraction onto specific wildlife host groups an effective method for detecting these ticks in the environment, even though it may be difficult to test collected individuals for pathogens. In this study we did not attempt to compare collection efficiency with another important group of tick hosts: large animals (i.e. *Odocoileus virginianus*, *Sus scrofa*, etc.). Large animals are important hosts for all stages of some tick species and for the adult stages of many tick species, making them another wildlife host group to consider when attempting to determine tick community composition in an area.

Using effective and efficient tick collection methods given a project's goals and resource limitations is an important way to improve risk assessments for humans and animals to ticks and tick-borne pathogens. Knowing which pathogen is targeted, the specific human-biting vector and relevant enzootic vectors, and the appropriate habitats and times of year to collect these tick species are all important factors to consider when selecting a tick collection method. The appropriate use of tick collection methods for active surveillance research will provide more accurate estimations of tick abundance, distribution, and habitat associations so that this information can be relayed into effective public health and animal management strategies.

Acknowledgements

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LITERATURE CITED

Addison, E., 2007. White as a Ghost, Winter Ticks and Moose. J. Wildl. Dis. 43, 1.

Anderson, B.E., Greene, C.E., Jones, D.C., Dawson, J.E., 1992a. Ehrlichia ewingii sp. nov., the etiologic agent of canine granulocytic ehrlichiosis. International journal of systematic bacteriology 42, 299-302.

Barbour, A.G., Maupin, G.O., Teltow, G.J., Carter, C.J., Piesman, J., 1996. Identification of an uncultivable Borrelia species in the hard tick Amblyomma americanum: Possible agent of a Lyme disease-like illness. J. Infect. Dis. 173, 403-409.

Bolker, B., Brooks, M., Gardner, B., Lennert, C., Minami, M., 2012. Owls example: a zero-inflated, generalized linear mixed model for count data.

Chong, S.T., Kim, H.C., Lee, I.Y., Kollars, T.M., Sancho, A.R., Sames, W.J., Klein, T.A., 2013b. Comparison of Dragging and Sweeping Methods for Collecting Ticks and Determining Their Seasonal Distributions for Various Habitats, Gyeonggi Province, Republic of Korea. Journal of Medical Entomology 50, 611-618.

Dantas-Torres, F., Lia, R.P., Capelli, G., Otranto, D., 2013. Efficiency of flagging and dragging for tick collection. Exp Appl Acarol 61, 119-127.

Dawson, J.E., Biggie, K.L., Warner, C.K., Cookson, K., Jenkins, S., Levine, J.F., Olson, J.G., 1996. Polymerase chain reaction evidence of Ehrlichia chaffeensis, an etiologic agent of human ehrlichiosis, in dogs from southeast Virginia. American journal of veterinary research 57, 1175-1179.

Eisen, R.J., Kugeler, K.J., Eisen, L., Beard, C.B., Paddock, C.D., 2017. Tick-Borne Zoonoses in the United States: Persistent and Emerging Threats to Human Health. ILAR J 58, 319-335.

Estrada-Pena, A., Gray, J.S., Kahl, O., Lane, R.S., Nijhof, A.M., 2013. Research on the ecology of ticks and tick-borne pathogens--methodological principles and caveats. Front Cell Infect Microbiol 3, 29.

Gherman, C.M., Mihalca, A.D., Dumitrache, M.O., Gyorke, A., Oroian, I., Sandor, M., Cozma, V., 2012. CO2 flagging - an improved method for the collection of questing ticks. Parasites Vectors 5, 7.

Ginsberg, H.S., Ewing, C.P., 1989. COMPARISON OF FLAGGING, WALKING, TRAPPING, AND COLLECTING FROM HOSTS AS SAMPLING METHODS FOR NORTHERN DEER TICKS, IXODES-DAMMINI, AND LONE-STAR TICKS, AMBLYOMMA-AMERICANUM (ACARI, IXODIDAE). Exp. Appl. Acarol. 7, 313-322.

Harrison, B.A., Rayburn, W.H., Toliver, M., Powell, E.E., Engber, B.R., Durden, L.A., Robbins, R.G., Prendergast, B.F., Whitt, P.B., 2010. Recent discovery of widespread Ixodes affinis (Acari: Ixodidae) distribution in North Carolina with implications for Lyme disease studies. J. Vector Ecol. 35, 174-179.

Keirans, J.E., Clifford, C.M., 1978. The genus Ixodes in the United States: a scanning electron microscope study and key to the adults. Journal of medical entomology. Supplement 2, 1-149.

Keirans, J.E., Durden, L.A., 1998. Illustrated key to nymphs of the tick genus Amblyomma (Acari: Ixodidae) found in the United States. J Med Entomol 35, 489-495.

Keirans, J.E., Litwak, T.R., 1989. Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi River. J Med Entomol 26, 435-448.

Labruna, M.B., Whitworth, T., Horta, M.C., Bouyer, D.H., McBride, J.W., Pinter, A., Popov, V., Gennari, S.M., Walker, D.H., 2004. Rickettsia species infecting Amblyomma cooperi ticks from an area in the state of São Paulo, Brazil, where Brazilian spotted fever is endemic. J. Clin. Microbiol. 42, 90-98.

Li, X.H., Dunley, J.E., 1998. Optimal sampling and spatial distribution of Ixodes pacificus, Dermacentor occidentalis and Dermacentor variabilis ticks (Acari : Ixodidae). Exp. Appl. Acarol. 22, 233-248.

Little, S.E., Dawson, J.E., Lockhart, J.M., Stallknecht, D.E., Warner, C.K., Davidson, W.R., 1997. Development and use of specific polymerase reaction for the detection of an organism resembling Ehrlichia sp. in white-tailed deer. J. Wildl. Dis. 33, 246-253.

Loftis, A.D., Mixson, T.R., Stromdahl, E.Y., Yabsley, M.J., Garrison, L.E., Williamson, P.C., Fitak, R.R., Fuerst, P.A., Kelly, D.J., Blount, K.W., 2008. Geographic distribution and genetic diversity of the Ehrlichia sp. from Panola Mountain in Amblyomma americanum. BMC Infect Dis 8, 54.

Mays, S.E., Houston, A.E., Fryxell, R.T.T., 2016a. Comparison of novel and conventional methods of trapping ixodid ticks in the southeastern USA. Medical and Veterinary Entomology 30, 123-134.

McCoy, K.D., Leger, E., Dietrich, M., 2013. Host specialization in ticks and transmission of tick-borne diseases: a review. Front. Cell. Infect. Microbiol. 3, 12.

Ouellette, J., Apperson, C.S., Howard, P., Evans, T.L., Levine, J.F., 1997. Tick-raccoon associations and the potential for Lyme disease spirochete transmission in the coastal plain of North Carolina. J Wildl Dis 33, 28-39.

Pfaff, M., James, A., Wehtje, M., Corn, J., Yabsley, M.J., Unpublished research. EVALUATING THE PHENOLOGY, HABITAT ASSOCIATIONS AND PATHOGEN PREVALENCE IN SELECTED SOUTHEASTERN TICK SPECIES. Ticks Tick-Borne Dis.

Ramos Vdo, N., Osava, C.F., Piovezan, U., Szabo, M.P., 2014. Complementary data on four methods for sampling free-living ticks in the Brazilian Pantanal. Rev Bras Parasitol Vet 23, 516-521.

Rulison, E.L., Kuczaj, I., Pang, G., Hickling, G.J., Tsao, J.I., Ginsberg, H.S., 2013. Flagging versus dragging as sampling methods for nymphal Ixodes scapularis (Acari: Ixodidae). J. Vector Ecol. 38, 163-167.

Rynkiewicz, E.C., Clay, K., 2014b. Tick community composition in Midwestern US habitats in relation to sampling method and environmental conditions. Exp Appl Acarol 64, 109-119.

Schulze, T.L., Jordan, R.A., Hung, R.W., 1997. Biases associated with several sampling methods used to estimate abundance of Ixodes scapularis and Amblyomma americanum (Acari : Ixodidae). Journal of Medical Entomology 34, 615-623.

Solberg, V.B., Neidhardt, K., Sardelis, M.R., Hildebrandt, C., Hoffmann, F.J., Boobar, L.R., 1992b. Quantitative Evaluation of Sampling Methods for Ixodes dammini and Amblyomma americanum (Acari: Ixodidae). Journal of Medical Entomology 29, 451-456.

Stromdahl, E.Y., Hickling, G.J., 2012. Beyond Lyme: aetiology of tick-borne human diseases with emphasis on the south-eastern United States. Zoonoses Public Health 59 Suppl 2, 48-64.

Terassini, F.A., Barbieri, F.S., Albuquerque, S., Szabo, M.P., Camargo, L.M., Labruna, M.B., 2010. Comparison of two methods for collecting free-living ticks in the Amazonian forest. Ticks Tick Borne Dis 1, 194-196.

Theuret, D.P., Trout Fryxell, R.T., 2018. Beefing Up Biosecurity: Survey of Ticks (Acari: Ixodidae) Currently Threatening the Tennessee Beef Cattle Industry, and a Proposed Monitoring Strategy for Invasive Ticks. Journal of Medical Entomology, tjy131-tjy131.

Tietjen, M., Esteve-Gassent, M.D., Medina, R.F., 2019. Searching for the Immature Stages of Ixodes scapularis (Acari: Ixodidae) in Leaf Litter and Soil in Texas. Journal of Medical Entomology 56, 35-39.

Wright, C.L., Sonenshine, D.E., Gaff, H.D., Hynes, W.L., 2015b. Rickettsia parkeri Transmission to Amblyomma americanum by Cofeeding with Amblyomma maculatum (Acari: Ixodidae) and Potential for Spillover. J Med Entomol 52, 1090-1095.

CHAPTER 4:
SUMMARY AND CONCLUSIONS

Tick communities vary across a number of different characteristics such species composition, relative abundance, as well as host associations and preferences. These qualities affect the likelihood that a person or domesticated animal will encounter a tick carrying a disease-causing pathogen. Determining risk to ticks and tick borne pathogens in an area rely upon surveillance methods to estimate tick community parameters. Active surveillance either through direct environmental sampling for ticks or indirect sampling of wildlife hosts represents an effective way to estimate these parameters. In our study comparing three of these active surveillance techniques, we found that the total number of ticks collected varied by collection method and across habitats and seasons. In total we collected 2,218 ticks of 8 species using 3 collection methods across 18 sites over the course of 4 seasons. Of these, 609 ticks of four species (558 *Amblyomma americanum*, 44 *Ixodes scapularis, 3 Amblyomma maculatum*, and 4 *Ixodes affinis*) were collected via tick drags. For CO^2-baited drags, 1089 ticks of four species (1029 *A. americanum*, 57 *I. scapularis*, 1 *Ixodes affinis*, and 2 *Dermacentor variabilis*) were collected. *A. americanum* larval clusters were recorded as a single tick for this study. Ticks collected off wildlife hosts included 520 individuals of seven species (125 *A. americanum*, 152 *Ixodes scapularis*, 121 *D. variabilis*, 45 *Ixodes texanus*, 73 *A. maculatum*, 3 *Haemaphysalis leporispalustris*, and 1 *Ixodes muris*). Significantly more ticks were collected with the CO^2-baited tick drag overall, with more ticks collected in the bottomland mixed hardwood forest habitats specifically and in the spring, summer, and winter field seasons. Wildlife or on-host tick

extractions collected significantly higher numbers of ticks only in the upland mixed hardwood forest habitat of the Piedmont physiographic region and in the fall season.

In addition, for two tick species which were collected using all three collection methods, number of individuals collected varied by collection method. For *Amblyomma americanum*, collection methods also varied by habitat and field season while collection methods only varied significantly by habitat for *Ixodes scapularis*. CO^2-baited tick drags collected significantly higher numbers of *Amblyomma americanum* at the bottomland mixed hardwood forest habitat types and in the spring field season but did not vary significantly from the other collection methods otherwise. Collection methods did not vary significantly in the total number of *Ixodes scapularis* collected, but they did vary in the number of ticks collected in the upland mixed hardwood forest habitat of the Piedmont physiographic region, in which wildlife tick extractions collected a greater number of individuals than the other collection methods.

Six pathogens that can cause disease in humans and animals were also screened for in environmentally-collected adult ticks. Infection prevalence for *Rickettsia* spp. in *A. americanum* adults tested was 85.53% and all sequenced PCR products were most closely related to *Rickettsia amblyommatis*. All 3 *A. maculatum* and both *D. variabilis* tested for *Rickettsia* spp. were positive, with the *A. maculatum* samples closely resembling *Rickettsia tamurae* (2/3) and the other *A. maculatum* sample closely aligning with the Boutonneuse fever fever agent: *Rickettsia parkeri*. The *D. variabilis* samples most closely resembled *Rickettsia amblyommatis*. All *A. maculatum* and *D. variabilis* ticks were negative for *Ehlrichia* spp.. *E. chaffeensis* infection prevalence in *A. americanum* was 5.35%, 5.66% for *E. ewingii*, and 3.77% for Panola Mountain *Ehrlichia* (Table 1.3).

Ixodes scapularis infection prevalence for *Borrelia* spp. was 2.02% and their sequences most closely aligned with *Borrelia burgdorferi*. Out of the 3 adult *Ixodes affinis* screened for *Borrelia* spp., all 3 were positive with *Borrelia burgdorferi* as well. For *Anaplasma phagocytophilum*, 3 *Ixodes scapularis* adults were positive (3.03%) and none of the *Ixodes affinis* adults were positive.

Total time and monetary investment varied by collection method, with more money and time spent on on-host tick extractions ($ 5,157.21 and 1112 hours) than both the traditional and CO^2-baited tick drags. When examined further, the majority of the time (9/15 site hours) and money ($53.04 / $71.63 per site) invested in on-host tick extractions came from mesomammal trapping efforts specifically, with rodent and passerine investments more closely resembling those of the traditional and CO^2-baited tick drags.

As is evident in these results, the tick communities estimated in a specific habitat vary by which tick collected method is used. If specific tick species are targeted, then the use of an efficient tick collection method for that species in the correct habitat type is recommended, but if multiple tick species are targeted then multiple tick collection methods are likely necessary to collect representative samples of each tick species in the community. Assuming that a single collection method employing a single activating stimulus is accurately representing multi-species or even single-species tick community diversity can lead to dangerously inaccurate conclusions that may impact public health interventions (Ginsberg and Ewing, 1989). Research on tick-pathogen ecology, and specifically on the influences of tick species co-feeding and the presence of enzootic vectors on pathogen prevalence, has highlighted the importance of assessing not only the prevalence of tick-borne pathogens in human-biting vectors, but also the presence and

pathogen prevalence of other tick species in these communities (Harrison et al., 2010; Ouellette et al., 1997; Wright et al., 2015b).

The employment of multiple tick collection methods does, however, come with the subsequent costs of deploying more equipment and spending more time at sample sites. One of the main reasons why the traditional tick drag is such a widely used tick collection method is because it is cheap, effective at collecting two tick species (*Ixodes scapularis* and *Amblyomma americanum*) which are important human-biting vectors, and takes little time to employ (Table 3.3). Using another stimulus such as carbon dioxide in the form of dry ice is slightly more expensive and time-consuming than simply conducting a tick drag, but it does collect significantly higher numbers of *A. americanum*, which may prove important for pathogen surveillance studies. The on-host tick extraction method collects the greatest species richness of all the tick collection methods compared here, thus better representing the tick community than the other two collection methods, but this method required a much higher investment of financial time resource. This method may be the only technique, however, to collect some important and infrequently environmentally detected species such as *Ixodes texanus*, *Ixodes muris*, and *Haemaphysalis leporispalustris*. On-host tick extraction also collected greater numbers of *D. variabilis* and *A. maculatum*, found on the mesomammal group and the rodent or bird groups respectively, than the other collection methods, making the targeting of on-host tick extraction onto specific wildlife host groups an effective method for detecting these ticks in the environment, even though it may be difficult to test collected individuals for pathogens. In this study we did not attempt to compare collection efficiency with another important group of tick hosts: large animals (i.e. *Odocoileus virginianus*, *Sus scrofa*, etc.). Large animals are important hosts for specialized tick species and for the adult stages of many tick species, making them

another wildlife host group to consider when attempting to determine tick community composition in an area.

Using effective and efficient tick collection methods given a project's goals and resource limitations is an important way to improve risk assessments for humans and animals to ticks and tick-borne pathogens. Knowing which pathogen is targeted, the specific human-biting vector and relevant enzootic vectors, and the appropriate habitats and times of year to collect these tick species are all important factors to consider when selecting a tick collection method. The appropriate use of tick collection methods for active surveillance research will provide more accurate estimations of tick abundance, distribution, and habitat associations so that this information can be relayed into effective public health and animal management strategies.

LITERATURE CITED

Ginsberg, H.S., Ewing, C.P., 1989. COMPARISON OF FLAGGING, WALKING, TRAPPING, AND COLLECTING FROM HOSTS AS SAMPLING METHODS FOR NORTHERN DEER TICKS, IXODES-DAMMINI, AND LONE-STAR TICKS, AMBLYOMMA-AMERICANUM (ACARI, IXODIDAE). Exp. Appl. Acarol. 7, 313-322.

Harrison, B.A., Rayburn, W.H., Toliver, M., Powell, E.E., Engber, B.R., Durden, L.A., Robbins, R.G., Prendergast, B.F., Whitt, P.B., 2010. Recent discovery of widespread Ixodes affinis (Acari: Ixodidae) distribution in North Carolina with implications for Lyme disease studies. J. Vector Ecol. 35, 174-179.

Ouellette, J., Apperson, C.S., Howard, P., Evans, T.L., Levine, J.F., 1997. Tick-raccoon associations and the potential for Lyme disease spirochete transmission in the coastal plain of North Carolina. J Wildl Dis 33, 28-39.

Wright, C.L., Sonenshine, D.E., Gaff, H.D., Hynes, W.L., 2015b. Rickettsia parkeri Transmission to Amblyomma americanum by Cofeeding with Amblyomma maculatum (Acari: Ixodidae) and Potential for Spillover. J Med Entomol 52, 1090-1095.