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Surveillance for Ticks and Tick-Borne Pathogens in Kentucky

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SURVEILLANCE FOR TICKS AND TICK-BORNE PATHOGENS IN KENTUCKY

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food and Environment
at the University of Kentucky

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2023

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ABSTRACT OF DISSERTATION

SURVEILLANCE FOR TICKS AND TICK-BORNE PATHOGENS IN KENTUCKY

Tick-borne diseases are an emerging threat to human and animal health. In Kentucky, tick-borne disease surveillance has identified rising incidences of spotted fever rickettsiosis, ehrlichiosis, and Lyme disease. Since these diseases occur through the bites of infected ticks, effective prevention efforts are reliant upon knowing where the risk of exposure to tick bites exists. Historical data on tick distribution in Kentucky is variable, with very little reported on a statewide scale, leaving vector control workers, public health personnel, physicians, veterinarians, and others to rely on outdated, intermittent, or out-of-state information. In my dissertation, I surveyed ticks and select tick-borne pathogens causing spotted fever rickettsiosis, ehrlichiosis, and Lyme disease in Kentucky from 2019-2022.

Chapter 1 reports data on *Ixodes scapularis* and *Borrelia burgdorferi*, the agent of Lyme disease. Six hundred and seventy-four *I. scapularis* were collected from 58 counties and the Lyme disease spirochete, *B. burgdorferi*, was detected in ticks from 16 of these counties adding to the few previous reports of *I. scapularis* and *B. burgdorferi* in Kentucky. This tick was collected each month of the year, though not every month of the study period, and primarily collected from forested environments.

Chapter 2 reports data on *Amblyomma americanum* and *Ehrlichia chaffeensis*, the primary agent of ehrlichiosis. Eight thousand forty-seven *A. americanum* were identified from 115 counties and *E. chaffeensis* was detected in ticks from 44 counties. This tick was collected most frequently in forested environments from March to November, with peak activity in May and June for adults and nymphs, and August for larvae.

Chapter 3 reports data on *Dermacentor variabilis* and *Amblyomma maculatum* infected with *Rickettsia rickettsii* and *R. parkeri*, both of which are agents of spotted fever rickettsiosis. One thousand one hundred seventy-six *D. variabilis* were collected from 99 counties, primarily in grassland dominant and mixed grassland-forest habitats. The Rocky Mountain spotted fever agent, *R. rickettsii*, was detected in ticks from three counties. Only 26 *A. maculatum* were collected from four counties, but no *R. parkeri* was detected in the ticks tested. This research is the first statewide, multi-year surveillance effort for ticks and

tick-borne pathogens in Kentucky. Overall, these data report on the distribution, abundance, and seasonality of these important tick vectors, and the distribution and estimated prevalence of pathogens causing major tick-borne diseases in Kentucky.

KEYWORDS: Tick surveillance, Tick-borne diseases, *Ixodes scapularis*, *Amblyomma americanum*, *Dermacentor variabilis*

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04/25/2023

Date

SURVEILLANCE FOR TICKS AND TICK-BORNE PATHOGENS IN KENTUCKY

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DEDICATION

To my family, Nora, Cass, Lonny, and Sock.

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CHAPTER 1. INTRODUCTION

Tick-borne diseases are an emerging threat to human and animal health. In Kentucky, the predominant causative pathogens are bacteria from the genera *Rickettsia*, *Ehrlichia*, and *Borrelia* causing spotted fever rickettsiosis, ehrlichiosis, and Lyme disease, respectively. A lack of local data on ticks and their associated pathogens has left vector control workers, public health personnel, physicians, veterinarians, and others to rely on outdated, intermittent, or out-of-state information. Here, I describe my research detailing tick distribution, abundance, and seasonality, and the presence and estimated prevalence of select tick-borne pathogens in Kentucky.

1.1 Tick biology and role as vectors

Ticks (order Ixodida) are small, blood-feeding ectoparasites that serve as vectors of disease-causing pathogens to humans and animals. Worldwide, there are over 800 species among 18 genera categorized into three families: *Nuttalliellidae*, *Argasidae*, and *Ixodidae*.

Nuttalliellidae consists of a single species, *Nuttalliella namaque*. This species is endemic to southern Africa and can be differentiated from the other two, more common, families by a combination of meticulous anatomical characteristics such as lack of setae, stigmata placement, integument structure, and formation of the spiracular plates (Roshdy et al., 1983). Ticks in the family of *Argasidae* occupy caves and burrows and, as such, do not normally encounter humans, instead feeding on other vertebrates (Donaldson et al., 2016). These ticks can be differentiated from those belonging to other families by the absence of the scutum, or shield, on their dorsal surface, earning them the common name of “soft ticks”. The genus of *Ornithodoros* within *Argasidae* is medically relevant for its

transmission of *Borrelia hermsii* and *B. turicatae*, which cause tick-borne relapsing fever in humans. Soft ticks may live upwards of ten years and take multiple blood meals during each stage of their life cycle.

Ixodidae is the largest family with over 700 species. Appropriately referred to as "hard ticks", they can be distinguished from the other families by the hard scutum on their dorsal surface and mouthparts that project forwards from the body (Estrada-Peña, 2015). Of all the families, Ixodidae are the most important in terms of public and veterinary health for causing illnesses such as Lyme disease, babesiosis, ehrlichiosis, Rocky Mountain spotted fever, anaplasmosis, and tularemia (Balashov, 1967; Estrada-Peña et al., 2008; Centers for Disease Control and Prevention f., 2022).

Ticks reproduce via internal fertilization, though there are a few exceptions of parthenogenetic species, namely *Haemaphysalis longicornis* (Kitaoka, 1961; Diehl et al., 1982; Matsuo et al., 2013). All ticks, regardless of family or reproductive status, develop through four life stages: egg, larva, nymph, and adult (Dennis & Piesman, 2005). Hard ticks feed once per life stage and lay a single batch of eggs after mating. Fertilized females lay eggs on the ground from which larvae hatch. Egg batches vary by species; *Dermacentor variabilis* lays an average of roughly 5,000 eggs, while *Hyalomma impeltatum* lays an average of 10,700 eggs (Sonenshine and Tigner, 1969; Logan et al., 1989). Larvae hatch with six legs, later gaining another pair as nymphs, and generally feed on mice and other small mammals, deemed "hosts". Fed larvae molt into nymphs, which take another blood meal before molting into adults. In most species, adult males do not feed but search for a female to mate. While adult male attachment has been documented, studies suggest this is likely not for nutritional reasons. By helping the female counterpart to feed, the male

maximizes his chance of mating and passing on his own genes, the fundamental goal of mate guarding (Wang et al., 1998). After reproducing, the adults die and the cycle repeats.

Depending on the species, *Ixodidae* ticks can have a one-host, two-host, or three-host life cycle. One-host life cycles are seen in species such as *Dermacentor albipictus, Rhipicephalus annulatus*, and *Boophilus microplus* (Sonenshine, 1991). These ticks remain attached and feed on the same host through all post-embryonic stages (i.e., larvae, nymph, and adult). Ticks with a two-host life cycle remain attached and feed on the same host as the larva and nymph, after which they drop off the host and find a place suitable to molt into adults. As adults, both males and females search for a new, often larger, host. *Hyalomma marginatum* is an example of a two-host tick (Sonenshine, 1991). Three-host life cycles are characteristics of more than 90% of ixodid species. These ticks feed on different hosts as larvae, nymphs, and adults. This feeding style permits the greatest chance of transmitting pathogens since agents can be vectored to and from multiple hosts.

Worldwide, hard ticks are the second leading cause of vector-borne disease in humans, although they remain the primary cause in the United States. They can transmit an array of pathogenic agents, including viruses, protozoa, and bacteria that cause disease in both humans and animals—some of which have no cure outside of supportive treatment (Corrin et al., 2018; Riccardi et al., 2019).

Ticks generally become infected with pathogens while feeding on infected hosts. If the tick can retain infection through its molts (i.e., transstadial transmission), then it may transmit those pathogens to its next host. Certain elements must be met for a pathogen to sustain infection in a vector and be successfully vectored further (e.g., attachment time, protein levels, gene expression requirements, and other host elements), leading to

specificity as certain tick species can only carry and transmit certain pathogens (Grimm et al., 2005; Couper et al., 2020).

Reservoir hosts are animals that harbor a pathogen without showing any ill effects and serve as a source of infection in the environment. Two model reservoir examples for many tick-borne pathogens are deer and mice (Lockhart et al 1997*;* Donahue et al., 1987). The availability of reservoirs to serve as hosts for vectors is critical to the survival of these pathogens. Similarly, these pathogens rely on the vector to multiply and spread to new hosts. Two- and three-host ticks fill this role since they parasitize more than one host in their lifetime, but one-host ticks are unlikely to effectively transmit pathogens to new hosts unless that pathogen can be passed on from the infected parent to offspring (i.e., transovarial transmission).

Dead-end hosts, on the other hand, do not facilitate the survival of pathogens. Humans are considered dead-end hosts for most arthropod-vectored pathogens since the agent cannot be transmitted to other susceptible hosts nor infect future vectors. This prevents the pathogen from completing its development. Generally, infection of dead-end hosts results in the development of disease (Baum, 2008). Infections caused by pathogens transmitted through a tick bite are known as tick-borne diseases.

1.2 Tick-borne diseases and tick vectors in Kentucky

Tick-borne disease cases in the United States have increased significantly in the last few decades (Centers for Disease Control and Prevention a, 2021). This is partly due to the expansion of tick populations in response to factors associated with climate change, host population shifts, and increases in urbanization and deforestation (Dumic & Severnini, 2018; Sonenshine, 2018; Gilbert, 2021; Ortiz et al., 2021). Several tick-borne diseases are

designated as nationally notifiable or reportable diseases. A reportable disease requires healthcare professionals, laboratories, hospitals, and other providers to report to their local public health department when an individual is diagnosed and includes personal information about the person and when they became ill. The diseases and conditions considered to be reportable are decided by the territory or state. Notifiable diseases are encouraged to be reported but are not required and do not include any personally identifiable information about the infected person(s). The Council of State and Territorial Epidemiologists and Centers for Disease Control and Prevention decide which diseases and conditions are reportable through an annual review. This system allows for case surveillance and data collection over time, which in turn provides information on which diseases are common, epidemiological trends, and risk factors for contracting the disease. The most commonly reported tick-borne diseases in Kentucky are spotted fever rickettsiosis, ehrlichiosis, and Lyme disease (Kentucky Department for Public Health 2021).

1.2.1 Spotted fever rickettsiosis

Rickettsial infections are caused by bacteria of the order *Rickettsia*les and are classically divided into either spotted fever group *Rickettsia* or typhus group *Rickettsia*. Spotted fever rickettsiosis is caused by bacteria of the spotted fever group rickettsia. The most serious and commonly reported spotted fever rickettsiosis in the United States is Rocky Mountain spotted fever, caused by *Rickettsia rickettsii*. When these bacteria enter a dead-end host, they multiply inside blood vessels and cause leakage into the surrounding tissue which, in turn, results in a characteristic spotted red rash. Early signs and symptoms include fever, head and muscle aches, gastrointestinal issues, vomiting, and the formation of an eschar at the bite site (Hackstadt, 1996). Historically, Rocky Mountain spotted fever

was considered a very severe disease with a reported fatality rate of nearly 25% in the 1940s. Fortunately, fatalities quickly declined with the availability of antibiotics. Today, the fatality rate is 0.5% of all cases (Centers for Disease Control and Prevention e, 2022).

Rickettsia rickettsii is maintained in a zoonotic cycle between the tick vector and host. A notable feature of this bacterium is its ability to persevere via transovarial, in addition to transstadial, transmission, allowing larvae, as well as nymphs and adults, to vector it. Because of this, there is less reliability on reservoir hosts to sustain the pathogen in nature. While *R. rickettsii* has been found in both domesticated (i.e., dogs) and wild animals, the understanding of whether they act as reservoirs or amplifying hosts is unclear (Bozeman et al., 1967; Bischof & Rogers, 2005).

Only three species of tick are known competent vectors of *R. rickettsii*. The primary vector in the United States is *D. variabilis*, the American dog tick, which occupies most of the eastern and central United States and southern Canada (Minigan et al., 2018). Secondary vectors include *Dermacentor andersoni,* the Rocky Mountain wood tick, which is a major vector in the Rocky Mountain region and parts of Canada, and *R. sanguineus*, the brown dog tick which is connected to transmission in Arizona (Demma et al., 2005; Dantas-Torres, 2007). Other tick vectors are known to transmit additional *Rickettsia spp.* that cause less severe forms of spotted fever rickettsiosis, specifically *Amblyomma maculatum* with *R. parkeri*.

Dermacentor variabilis is a pervasive tick commonly found in fields and open woodlands as opposed to dense forests (Bishop & Trembley, 1945; Burg, 2001). This species is one of the most widely distributed ticks in the United States, but certain climate tolerances (i.e., humidity) restricts it to the east of the Rocky Mountains (Bishop &

Trembley, 1945; James et al., 2015). Though there exists an isolated population along the west coast concentrated in California, recent taxonomic studies have described this as a separate species, *D. similis* (Lado et al., 2021). Generally, this species is active in the spring and summer months with adults acting as the main life stage to parasitize humans (Guglielmone & Robbins, 2018). While this tick remains the species primarily responsible for Rocky Mountain Spotted fever transmission, laboratory studies have revealed that infections levels of questing *D. variabilis* by *R. rickettsii* are very low (Sonenshine & Mather, 1994; Stromdahl et al., 2011; Fritzen et al., 2011).

Rocky Mountain spotted fever was first declared a notifiable disease in the 1920s. At the turn of the 21st century, less than 500 cases were reported nationwide with cases more than tripling over the following decade. In 2008, 2,557 human cases of Rocky Mountain spotted fever were reported (Centers for Disease Control and Prevention c, 2019). Then, in 2010 the case definition was changed to recognize *R. rickettsii* infections under a new category called Spotted Fever Rickettsiosis due to the inability to distinguish between different spotted fever group rickettsial infections in humans. The increase in human case counts continued and in 2018, 5,544 human cases of spotted fever rickettsiosis were documented, a 180% nationwide increase since the case definition change in 2010 (Centers for Disease Control and Prevention c, 2019).

Cases of spotted fever rickettsiosis are largely concentrated in the southeastern United States. In fact, while human cases are reported from each of the lower 48 states, over half of all reported cases come from five states: Arkansas, Missouri, North Carolina, Tennessee, and Virginia. Even so, Kentucky is regularly recognized as a high-incidence state for this disease. In 2018, Kentucky's annual incidence was 48.87 cases per million

persons, which was higher than incidences reported for 42 other states (Centers for Disease Control and Prevention e, 2022). This put Kentucky in the top 16% for human cases nationwide that year, while it was ranked in the top 16%, 14%, and 20% for the three years preceding.

Of course, due to the merging of Rocky Mountain spotted fever under the spotted fever group rickettsiosis umbrella it is impossible to report the exact number of *R. rickettsii* infections that have occurred. The lack of pathogen detection in *D. variabilis* has led to questions regarding how such low levels of infection in the vector could result in so many reports of human cases. A widely shared theory postulates that previous reports of *R. rickettsii* infections—Rocky Mountain spotted fever—may have been misdiagnosed and were, in fact, caused by a different *Rickettsia spp.* infection. Most likely, *R. parkeri*.

Until 2002, Rocky Mountain spotted fever was the only known tick-borne spotted fever in the United States. Then, *R. parkeri* was implicated as a secondary agent of rickettsiosis when a Virginia man presented with symptoms similar to Rocky Mountain, but sequences from the biopsy specimen identified *R. parkeri* as the match for each gene target examined, providing concrete evidence that this bacterium was able to cause human disease (Paddock et al 2004). The illness became known as *R. parkeri* rickettsiosis. Since clinical symptoms are alike and serologic tests fail to differentiate between the two bacteria, there is virtually no way to diagnose Rocky Mountain spotted fever from *R. parkeri* rickettsiosis.

Rickettsia parkeri rickettsiosis is strictly vectored by *A. maculatum*, the Gulf Coast tick. This tick was historically present in the southern United States near the Gulf Coast region, but expansion via host transport has carried it further northward (Paddock &

Goddard, 2015). Nadolny & Gaff 2018 identified predictors of *A. maculatum* presence over a five-year study period in nearby Virginia, finding that the Gulf coast tick had habitat preferences similar to *D. variabilis* and that the co-establishment of *D. variabilis* significantly increased the presence of *A. maculatum. Rickettsia. parkeri* infection in *A. maculatum* is much higher than that of *R. rickettsii* in *D. variabilis*, thus exposure to the former is presumably more likely than the latter (Sumner et al., 2007; Pagac et al., 2014). Combined with the extreme clinical similarities between these two diseases, the misdiagnosis of *R. parkeri* rickettsiosis as Rocky Mountain spotted fever is entirely plausible and better matches the narrative conveyed by infection status in the vector. Surveillance for these pathogens in their respective tick vectors could provide useful information for predicting *R. parkeri* and *R. rickettsii* prevalence, therefore providing some direction as to which rickettsiosis persons are more at risk for.

1.2.2 Ehrlichiosis

Ehrlichiosis is caused by bacteria of the genera *Ehrlichia* and *Anaplasma* in the family *Anaplasmataceae*. In humans, *Ehrlichia chaffeensis* is the most common cause of illness. When this bacterium enters the body, it infects blood leukocytes and causes low levels of white blood cells and platelets. While the illness is often mild, it can progress and be fatal if left untreated. Common symptoms of ehrlichiosis include fever, aches, chills, vomiting, and gastrointestinal distress (Paddock & Childs, 2003). Roughly 30% of infected adults and 60% of infected children will develop a rash (Paddock & Childs, 2003). More severe symptoms include nervous system damage, respiratory failure, internal bleeding, and organ failure (Centers for Disease Control and Prevention a, 2019).

Ehrlichia chaffeensis is maintained through a zoonotic cycle. The main reservoir host is *Odocoileus virginianus*, the white-tailed deer, which is naturally infected with the bacteria in the southeastern United States (Lockhart et al 1997; Davidson et al., 2001). Other vertebrate reservoirs include coyotes, raccoons, opossums, and domesticated dogs (Lockhart et al 1997; Kocan et al., 2000; Yabsley, 2010). Transovarial transmission has not been documented, thus unfed larvae must first feed on an infected host to become a vector. Once infected, the tick may transmit the pathogen to other reservoir hosts or humans during blood feeding (Paddock & Childs, 2003).

The tick responsible for *E. chaffeensis* transmission is the lone star tick, *Amblyomma americanum*. All life stages are known to parasitize humans and they can be found in large abundances in a wide variety of habitats, although it is primarily a woodland-associated species (Kollars et al., 2000; Springer et al., 2015). Several studies have documented its rapid rate of expansion northward, which is largely accredited to increases in the range and abundance of white-tailed deer, their primary host (Childs & Paddock, 2003). This tick is notorious for its aggressive, non-specific biting habits and is the most common tick to bite humans in the southeastern United States (Stromdahl and Hickling 2012; Gaines et al., 2014).

Ehrlichiosis was first recognized as a human disease in the United States in the 1980s but was not classified as a notifiable disease until 1999. Data was first recorded in the year 2000, with 201 cases reported across the nation (Centers for Disease Control and Prevention c, 2019). In 2008, the case definition was split into four categories allowing public health officials to report *E. chaffeensis*-caused ehrlichiosis separate from the other human forms (*E. ewingii and E. muris eauclairensis ehrlichiosis)*, 962 cases were reported

that year. Finally, in 2018, 1,799 cases were reported: a 795.0% nationwide increase since the first year of record (Centers for Disease Control and Prevention c, 2019).

In 2018, Kentucky's annual incidence was 16.81 cases per million persons. Kentucky was in the top 18% for human cases that year nationwide, and the top 14%, 18%, and 14% for the years preceding (Centers for Disease Control and Prevention c, 2022). Kentucky is considered a high-incidence state for ehrlichiosis. Reports from public health departments document disease across the state, but human cases are specifically concentrated in the western and southeastern regions (Kentucky Department for Public Health 2021). This disease is also seen in high incidences among domesticated canines. According to the Companion Animal Parasite Council, nearly 3% of all canines tested nationwide are positive for an ehrlichiosis infection. The infection rate among canines in Kentucky is 6.91% (Companion Animal Parasite Council 2012).

Since *E. chaffeensis* ehrlichiosis is only noted to be vectored by *A. americanum*, one can confidently theorize that the increase in human and animal cases is tied to the rise in lone star tick abundance. While several studies document *A. americanum* presence in Kentucky (Mount & Snoddy 1983; Goddard & Norment 1986; Fritzen et al. 2011; Lockwood et al, 2018; Slabach et al 2018), no study has reported on its distribution statewide over a multi-year study period. This prevents knowing where this tick is established in the state, and how quickly the population is growing and expanding.

1.2.3 Lyme disease

Lyme disease is the most common tick-borne disease in the United States. It is caused by *Borrelia*-genus bacteria with *B. burgdorferi* sensu stricto as the primary causative agent in North America. Two additional species, *B. garinii,* and *B. afzelli*, are

responsible for disease in Europe and Asia. This bacterium can only be spread through the bites of infected *Ixodes* ticks (Wolcott et al., 2021). When the spirochetal bacteria enter the body, they multiply and spread outwards, later entering the bloodstream, and spreading to the joints, heart, and nervous system to cause various symptoms. Host inflammatory responses in the skin cause the diagnostic erythema migrans (EM) rash. (Tugwell et al., 1997).

Lyme disease can be characterized into three categories of infection. Early localized infection occurs prior to the bacteria's dissemination through the body. It includes symptoms such as the development of an EM rash at the bite site, fatigue, aches, fever, and chills (Shapiro 2014). Early disseminated infection occurs after dissemination in the body and may include the formation of additional EM rashes across the body, transient aches, and joint swelling (Wright et al 2012). Late-stage infection is more serious and can manifest as arthritis, facial palsy, inflammation of the brain, nerve pain, and the development of Lyme carditis (Wright et al., 2012; Centers for Disease Control and Prevention b, 2021).

Borrelia burgdorferi circulates between *Ixodes* ticks and reservoir hosts. The primary reservoir in the United States is *Peromyscus leucopus*, the white-footed mouse (Donahue et al., 1987). Other small mammals and birds serve as additional reservoir hosts, with reptiles being competent but poor reservoirs (Anderson & Magnarelli, 1984; Levin et al., 1996; Clark et al., 2005). The bacteria are rarely transmitted transovarially; thus, it is generally assumed that larvae are uninfected (Patrican 1997; Tilly et al., 2008; Rollend et al., 2013). Nymphs and adults, if infected, may transmit the pathogen to humans and other hosts.

Ixodes scapularis, commonly referred to as the blacklegged or deer tick, is the primary vector of Lyme disease in the United States. *Ixodes pacificus* is another, less accountable, vector on the west coast, while other *Ixodes* species are responsible for transmission outside of the U.S. (Re et al., 2004). *Ixodes scapularis* is found in the eastern United States and northern Midwest up into Canada. It develops through a two-year life cycle where larvae hatch in late spring and summer, molt over the winter, emerge as nymphs in the spring, then molt and emerge as adults in the late fall and following winter (Yuval & Spielman 1990). Initial studies report low abundances of this species outside of the northeast, but more recent findings support a much larger range of suitable habitats than previously thought (Guerra et al., 2002; Johnson et al., 2016; Hahn et al., 2016).

Cases of human Lyme disease have increased significantly since the last century. It was first designated as a nationally notifiable disease in 1991 with 9,909 human cases of Lyme disease reported to the Centers for Disease Control and Prevention in 1992. By 1998, case reports had increased to 16,802 (Centers for Disease Control and Prevention, 2000). Ten years later, cases more than doubled when 35,198 cases were reported (Schwartz et al., 2017). By 2018, the percent increase in human Lyme disease cases from the first year of record, 1992, was 239.8% (Centers for Disease Control and Prevention c, 2019). In that same time frame, eight new tick-borne pathogens were identified as causes of human disease, including *B. mayonii*, a secondary agent of Lyme disease (Centers for Disease Control and Prevention b, 2019). Of all the tick-borne cases reported to the Centers for Disease Control and Prevention that year, 33,666 of the 47,743 (70.5%) were Lyme disease cases (Centers for Disease Control and Prevention c, 2019).

While Lyme disease is the most common tick-borne disease in the nation, it is highly concentrated in the northeastern United States, with increasing, but sporadic cases in the south. According to prevailing theories, the higher incidence of Lyme disease in northern areas may be due to ticks parasitizing highly effective reservoir hosts like mice and shrews, while feeding on less effective reptilian hosts in the south. Additionally, there may be behavioral differences between the northern and southern populations of these ticks that impact the risk of human exposure, as well as lower the overall abundance of the species in the south (Xu et al., 2020; Ginsberg et al., 2021). Like much of the southeast, Kentucky is considered a low-incidence state for human Lyme disease, although the number of cases is gradually increasing (Centers for Disease Control and Prevention d, 2022). The known presence of *I. scapularis* in Kentucky in 2018 was poor due to a lack of surveillance, with few published studies and no statewide reports (Dennis et al., 1998; Eisen et al., 2016; Buchholz et al., 2018; Lockwood et al., 2018; Slabach et al., 2018). As a result, insufficient understandings of *Ixodes* exposure and subsequent Lyme disease exposure in Kentucky were weak and caused domino effects within the healthcare system. Because Lyme disease had not been previously identified in the state and there was limited data on the presence of and transmission by the tick vector, many people held the mistaken belief that the disease was not a concern in Kentucky. This misconception put patients at risk of misdiagnosis, including a higher likelihood of false positives, which are more common in regions where the disease is rare (Mead, 2015). Given that Lyme disease is known to have chronic effects on untreated patients, early recognition, diagnosis, and treatment by physicians are important to prevent long-term illness.

In summary, human cases of these tick-borne diseases—which are the most commonly reported in Kentucky—are increasing. Human tick-borne disease surveillance led by the Kentucky Department for Public Health has been ongoing for several years in accordance with CDC's regulations for reportable and notifiable diseases. However, surveillance for ticks and the pathogens within them has not been appropriately performed. While past studies have documented repeated findings of tick vectors and the pathogens responsible for these illnesses, no long-term research has been performed and nothing is reported on a statewide scale—leaving some regions without any data at all.

1.3 Impacts of tick and tick-borne pathogen surveillance

A statewide surveillance program for ticks and their associated pathogens would provide data with multidisciplinary impacts. Data can be used by those in pest control, public health, and veterinary science for accurate control, prevention, and treatment of ticks and diseases. Within such a surveillance program, the implementation of both active and passive collection methods is best. Active surveillance provides data on questing tick populations, naturally occurring pathogens, population densities, and habitat and climate trends, while passive surveillance acts as a cost-effective and wide-ranging approach for understanding host interactions, population statuses, occupational and recreation risks, and provides an avenue to communicate prevention practices to the public (National Center for Emerging and Zoonotic Infectious Diseases (U.S.) Division of Vector-Borne Diseases Bacterial Diseases Branch 2019, 2020 ; Lyons et al. 2021).

1.3.1 Vector control impacts

To ensure the effectiveness of a tick control method, it is essential to understand factors such as seasonal distributions, host preferences, and habitat associations. This understanding is critical because a control method specifically tailored to the current scenario is often the most effective approach. Active surveillance can provide valuable data on environmental factors influencing tick activity, as well as population densities and host associations. Armed with this information, one can determine the best management practices for controlling tick populations (Centers for Disease Control and Prevention, 2020).

Data on habitat preferences of local tick populations is helpful in determining what species may be likely to establish on farms, in forested parks, etc., and therefore aids in control. For example, agricultural land—particularly that which houses livestock—is largely unshaded to allow for sufficient growth of grass for grazing animals. While this ecosystem may not be particularly suitable for forest-dwelling species like *I. scapularis*, it is preferable to *D. variabilis* and *A. maculatum* which prefer open fields and grasslands (Bishop & Trembley, 1945; Burg, 2001; Teel et al., 2010; James et al., 2015; Nadolny & Gaff, 2018). From this, an appropriate control recommendation would be the use of acaricides that are effective against *Dermacentor* and *Amblyomma spp.* and education about the signs and symptoms of relevant tick-borne diseases in livestock. The same ideology can be applied to public parks, campgrounds, outdoor recreation areas, suburban neighborhoods, and more.

1.3.2 Public health impacts

Surveillance for tick-borne diseases provides critically important information to identify where cases occur. Tick surveillance complements tick-borne disease surveillance by providing estimates of the distribution and abundance of tick vectors, as well as the presence and prevalence of associated pathogens in those ticks. This data can be useful in many corners of public health such as identifying when and where persons are at risk for exposure to ticks and tick-borne pathogens, aiding in the explanation of current disease trends, predicting future trends, and discovering novel threats from species or pathogens.

Surveillance provides several avenues for extension and education opportunities, too. Tick-borne diseases are transmitted to humans through the bite of an infected tick; thus, exposure to ticks is the fundamental component that determines the disease risk. Simply put: if you can avoid getting bit, you can avoid getting sick. Factors such as land use patterns, suburban development, and changing climate patterns have all been linked with the expansion of, and increased exposure to, ticks and disease (Raghavan et al 2019; MacDonald et al. 2020; Alkishe et al. 2021; Gilbert 2021; Noden et al. 2022). Certain outdoor occupations and recreational activities also increase exposure to tick bites. Studies suggest that outdoor workers are at risk of being diagnosed with a tick-borne disease roughly 3–10 times more so than those with indoor occupations (Smith et al. 1988; Schwartz & Goldstein 1990; Donohoe et al 2018; Bellamy et al 2019). Although these risks seem common sense throughout the agricultural and healthcare communities, data on the extent of risk in areas where agriculture work persists is few, and the knowledge of recognizing, testing, and diagnosing tick-borne diseases among the medical community of Kentucky is inconsistent. This undoubtedly contributes to the presumed notion that tick-

borne diseases are vastly underreported, which hampers the ability to accurately monitor and track disease growth (Young 1998; Madison-Antenucci et al. 2020). Additionally, the high cost of testing for tick-borne disease and delay in seeking care by agricultural workers, particularly farmers, compounds the issue (Mobed et al. 1992; Reed et all 2008). Since several tick-borne diseases progress rapidly and require swift treatment, early recognition and care of infection by both patient and practitioner is an essential step in preventing serious illness and/or fatality.

1.3.3 Veterinary health impacts

The use of passive surveillance systems, in particular, can be incredibly useful for understanding tick-host dynamics among livestock and domesticated animals. In many countries, sentinel animals are commonly used to detect risks to human health by monitoring the prevalence of zoonotic diseases. In the United States, for example, *Yersinia pestis*, the agent responsible for the plague, is monitored through sentinel animal infection status (Bevins et al., 2012). Additionally, studies on canine seroprevalence have supported the use of dogs as sentinels to help characterize the risk of Lyme disease in humans (Duncan et al., 2005). While sentinel animal surveillance is helpful, surveillance for the pathogen in the vector can provide risk insight before infection in any hosts.

While some tick species are important vectors of pathogens, such as *H. longicornis* and *Theileria orientalis*, others can have detrimental impacts aside from pathogen transmission; *D. albipictus* and the invasive *H. longicornis*¸ have both demonstrated severe—even fatal—effects on hosts by exsanguination, induced hair loss, and anemia on hosts in the United States (Hoogstraal et al. 1968; Heath 2016; Jones et al. 2019; Dinkel et al. 2021). Agriculture is one of Kentucky's leading and most essential industries with the

largest cattle industry east of the Mississippi River, contributing roughly $45.6 billion to Kentucky's economy every year according to the 2017 Census of Agriculture (United States Department of Agriculture 2019). Maintaining a healthy cattle population is crucial, so any disease outbreaks would cause substantial losses to the industry and economy through decreased productivity, working efficiency, and increased cost for control measures (Uilenberg 1995; Zintl et al. 2003; Gilbert 2016). Therefore, protecting and enhancing the health of livestock is important to the state's economy, and implementing tick-borne pathogen surveillance on farms and stockyards could reveal threats before infection occurs.

1.4 Summary

The prevention of tick-borne diseases relies heavily on an accurate understanding of when and where persons are at risk for exposure to ticks and the pathogens they transmit. However, predicting these risks can be tricky, especially without the availability of up-to-date data. The primary obstacle that prevents effective tick-borne disease mitigation in Kentucky is the lack of knowledge about local tick and pathogen populations, hence the need for a well-established, large-scale surveillance effort for ticks and their associated pathogens. As of 2018, an effort to perform tick and tick-borne pathogen surveillance statewide had not been made, despite increasing disease cases over the last two decades. This, in turn, has forced reliance on outdated, intermittent, and out-of-state data.

Based on previous studies documenting tick activity in Kentucky, the most commonly reported species are *A. americanum, D. variabilis,* and *I. scapularis*. Coincidentally, these three species act as the primary vectors of the three most commonly reported tick-borne diseases in Kentucky. As climatic and anthropogenic factors

influencing tick activity increase, a paralleled increase in tick-borne disease cases is occurring (Guerra et al., 2002: Gray et al., 2009; Hasle, 2013; Springer et al., 2015; Dumic & Severnini, 2018; Minigan et al., 2018; Sonenshine, 2018; Raghavan et al., 2019; MacDonald et al., 2020; Alkishe et al., 2021; Gilbert, 2021; Ortiz et al., 2021; Noden et al., 2022). The research presented here aims to determine the distribution, abundance, and seasonality of tick vectors, and the presence and estimated prevalence of select tick-borne pathogens in Kentucky. Particularly, it aims to investigate the dynamics of 1) *I. scapularis* and *B. burgdorferi*, 2) *A. americanum* and *E. chaffeensis*, and 3) *D. variabilis* and *R. rickettsii*, and *A. maculatum* and *R. parkeri*. The data produced in this research will be useful for updating records on tick and tick-borne pathogen presence in the state but will also have impacts across fields of vector control, public health, and veterinary health.

CHAPTER 2. MAPPING DISTRIBUTIONS OF THE LYME DISEASE VECTOR, *IXODES SCAPULARIS*, AND SPIROCHETE, *BORRELIA BURGDORFERI*, IN KENTUCKY USING PASSIVE AND ACTIVE SURVEILLANCE

This chapter contains data published in an academic journal (Pasternak & Palli, 2022) but has been updated to include data from the years 2021 and 2022 that have not been published.

2.1 Abstract

Lyme disease, the most common tick-borne illness in the United States, is becoming more prevalent each year. The disease is transmitted through the bites of ticks infected with *Borrelia burgdorferi* and generally includes *Ixodes scapularis* in the eastern United States, *I. pacificus* in the western U.S., and *I. ricinus* in Europe and Asia. Despite not being endemic in Kentucky, the number of reported human Lyme disease cases in the state has increased dramatically in recent years; In 2010, there were only five reported cases, while in 2019, that number increased by over 300%. To better understand the distribution of *I. scapularis* populations infected with *B. burgdorferi*, effective management programs for ticks and diseases must be developed, and the first step in this process is monitoring disease spread. To that end, in collaboration with the Kentucky Department for Public Health, active and passive surveillance methods were used to collect 674 *I. scapularis* ticks from 58 counties in Kentucky between March 2019 and December 2022. Subsequent testing revealed the presence of *B. burgdorferi* in tick populations from 16 counties, with a minimum infection rate of 4.46% across the entire study period. The results of this study highlight the importance of surveillance in monitoring the growing risk of Lyme disease in states such as Kentucky, where the incidence was previously low. By

adding to the limited data on *I. scapularis* and *B. burgdorferi* distribution in Kentucky, this research provides a crucial foundation for future studies and management efforts.

KEYWORDS: Lyme disease, *Ixodes scapularis,* vector surveillance, tick-borne disease, ticks

2.2 Introduction

Recently, reported cases of several tick-borne diseases have reached record levels. Lyme disease cases in the United States have increased significantly since the late 1990s. The number of reported human cases of Lyme disease to the CDC more than doubled from 16,455 in 1996 to 34,945 in 2019 (Centers for Disease Control and Prevention, 1997). However, this figure is believed to be a significant underestimation of the true number of cases. The CDC estimates that approximately 476,000 individuals are treated for Lyme disease annually in the United States, indicating that the actual number of cases is likely much higher than reported (Kugeler et al., 2021; Schwartz et al., 2021).

The causative agent of Lyme disease is *Borrelia burgdorferi* sensu stricto (hereafter deemed *B. burgdorferi*) in the United States. It exists in nature by cycling between natural reservoirs, most notably white-footed mice and ixodid ticks, which act as a vector to other hosts. When the bacteria enter a human host, they often result in the development of Lyme disease. Symptoms include fever, chills, head and body aches, fatigue, and the development of an erythema migrans (EM) rash (Stanek et al., 2012; Shapiro, 2014). If left untreated, the disease may progress to trigger neurological issues, cardiac complications, and arthritis (Cardenas-de la Garza et al., 2019). In dogs, this disease can manifest as arthritis, fever, joint swelling, lameness, lethargy, lymphadenopathy, and, rarely, nephropathy (Littman, 2013; Bouchard et al., 2015).

Lyme disease is the most common tick-borne disease in the United States. Its rising incidence in the eastern U.S. has been linked to the growing density of *Ixodes scapularis* populations (Eisen et al., 2018; Hickling et al., 2018). Historical records suggest that this species originated in the Southeast and later expanded north with the earliest documentation in the northeast from a collection in Massachusetts during the 1920s (Van

Zee et al., 2015). By the 1940s, *I. scapularis* was collected intermittently along the northeastern coastline but remained abundant in southeastern and Gulf Coast states (Dennis et al., 1998). Over the next 50 years, expansion persisted until the vector was found along the entire east coast (Spielman et al., 1985; Arsnoe et al., 2019). Today, surveillance finds abundant populations of this species in the northeastern United States and fewer, although increasing, populations in areas of the Midwest and Southeast, alluding to a southward expansion of the northeastern populations (Brownstein et al., 2005; Eisen & Eisen, 2018; Gardner et al., 2020). Since northern populations of *I. scapularis* pose a greater threat of biting humans and transmitting *B. burgdorferi*, this reintroduction is of great public health concern (Arsnoe et al., 2019; Ginsberg et al., 2021). Transportation into these regions is likely occurring on hosts, such as migratory birds, white-tailed deer, and domesticated pets (Scott et al., 2012; Lommano et al., 2014; Roome et al., 2017), while traditional southeastern environmental factors such as high humidity, frequent precipitation, and dense ground cover influence establishment (Guerra et al., 2002; Gardner et al., 2020; Telford et al., 2017).

Kentucky regularly reports low annual incidences of Lyme disease—an average of 0.2 cases per 100,000 persons—although West Virginia and Virginia report 32.5 and 10.1 incidence rates, respectively (Centers for Disease Control and Prevention d, 2022). While Lyme disease is not as common as other tick-borne illnesses in Kentucky, the number of cases reported by the state health department has gradually increased over the past decade (Centers for Disease Control and Prevention a, 2021). Potential explanations for the underreporting of Lyme disease include the cost of testing, a lack of knowledge among medical communities, and difficulties with diagnosis. To ensure that physicians and health

officials understand the local risk of Lyme disease and other tick-borne illnesses, state surveillance and diagnostic testing is required, especially since vector and pathogen dynamics vary by state.

The dynamics of *I. scapularis* and *B. burgdorferi* in Kentucky is of particular interest since this area represents a unique geolocation standing between states of high and low incidence for Lyme disease. Previous studies on the establishment of *I. scapularis* in Kentucky identified populations sporadically across the state, with detections of *B. burgdorferi* in a few of these populations (Dennis et al., 1998; Eisen et al., 2016; Lockwood et al., 2018). However, the number of published studies on this tick and pathogen in Kentucky remains limited because of the low survey effort in the state. Effective public health strategies to prevent Lyme and other tick-borne diseases rely on the availability of accurate information about vector and pathogen distribution. Therefore, I set out to perform statewide, multi-year surveillance to collect the necessary data.

Here, I report on my findings of *I. scapularis* and *B. burgdorferi* distribution in Kentucky counties from 2019 to 2022. The objectives were to 1) determine where *I. scapularis* populations were present and 2) if *B. burgdorferi* was present in those tick populations. I utilized passive surveillance in the form of a tick submission program to attempt data collection from as many Kentucky counties as possible, as repeated collections in all counties were not practical. Since the study objective was determining the presence/absence of both this tick and pathogen, the use of these two methods together was sufficient as outlined in the CDC's "Guide to the Surveillance for *I. scapularis* and pathogens found in this tick species in the United States" (National Center for Emerging and Zoonotic Infectious Disease, 2019).

2.3 Materials and Methods

2.3.1 Active surveillance

Tick collection occurred from March 2019 through December 2022. Sampling locations were chosen based on the presence of suitable tick habitats described in previous studies (Guerra et al., 2002; Ginsberg et al., 2020). Sampling sites where ticks were collected included hiking trails, wildlife management areas, and other publicly accessible recreation areas. If an appropriate public site could not be accessed, collections occurred on private land with the owner's permission.

Ixodes scapularis was collected from the environment using the drag-cloth method. In this, a one-meter by one-meter light-colored cloth attached to a dowel rod is pulled along the ground at a slow pace by an attached rope (Figure 2.1). All ticks were removed from drags and placed in vials containing 70% ethanol for transport back to the laboratory. At the time and location of each collection event, GPS coordinates, temperature, and habitat type (forest, brush, grassland, forest and brush, forest and grassland, or brush and grassland) were recorded. All ticks were returned to the laboratory at the University of Kentucky and stored at -20C^{o} until identification.

2.3.2 Passive surveillance

Veterinary practices in all 120 of Kentucky's counties were invited to submit ticks removed from animals brought into their clinic between March 2019 through December 2022 as part of the Kentucky Veterinarian Tick Submission Program. Participants were provided instructions on how to package and ship the tick for submission and filled out a form to collect basic contact information as well as the date of collection, county of collection, host species, age, and breed, travel outside the home county in the two weeks preceding, the use of tick preventative medicine (if applicable), habitat type in which the

tick was collected and the site ownership type (Figure 2.2). All specimens were shipped to the University of Kentucky Department of Entomology.

In addition, I accepted ticks from non-veterinary practices, including health departments, physician offices, and the public. With each submission, information forms were included to obtain basic contact information, the date the tick was collected and shipped, county of collection, travel outside the home county in the two weeks preceding, habitat type in which the tick was believed to be collected, site ownership type, host type, and whether the tick was attached or not (Figure 2.3). Directions on packaging and shipping the tick were also included. Samples from outside Kentucky or instances when the date and/or county-level location information could not be provided were not accepted. All ticks received through these submission programs were stored under the same conditions as ticks collected from the environment.

2.3.3 Tick identification

Ticks were identified and sexed morphologically by comparison with standard keys in the laboratory at the University of Kentucky (Keirans & Litwak, 1989). Ticks that were identified as anything other than the target organism, *I. scapularis*, were omitted from this chapter and included in other chapters.

2.3.4 DNA Extraction

For DNA extraction, individual ticks were bead-beaten with 2.0 mm Zirconia beads from BioSpec Products in a Tissueminser (MP Biomedicals) at a speed of 0.6 m/s for three consecutive cycles of 40 s each to ensure proper lysis. The homogenate for each tick in the sample was then pooled. DNA extraction was performed using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturer's instructions. Since the objective was to

determine the presence/absence of the pathogens, I pooled ticks for testing. Ticks were not combined from multiple submissions/collection events, hosts, or locations for testing. Individual tick testing was not performed. When testing, no more than five adults were used per sample. Nymphs were pooled regardless of number. Larvae were not tested as they are not known to transmit *B. burgdorferi.* The remaining homogenate was stored at -20ºC for future study.

2.3.5 Detection of *B. burgdorferi*

Detection of *B. burgdorferi* employed the use of primers targeting the *dbpA* gene with dbpA-F (GGTATCAGAAAATCCATTCATACTTG) and dbpA-R (TACATTGCTGAAAATTCACCACTACTT) primers (Wroblewski et al., 2017)**.** Each qPCR run included one positive control of DNA confirmed to be infected with the target pathogen and two negative controls containing non-target DNA and no DNA. Reactions containing 5 uL SYBR Green Master Mix (Bio-Rad), 2 uL extracted DNA, 0.5 uL of forward primer, 0.5 uL of reverse primer, and 2 uL nuclease-free water were run on Bio-Rad iCycler at the following settings: one cycle of 95ºC for five minutes, 45 cycles of 95ºC for 15 seconds, and 55ºC for 30 seconds.

2.3.6 Determination of county establishment status

In accordance with the "Guide to the Surveillance for *I. scapularis* and pathogens found in this tick species in the United States", I classified county status based on the county-level establishment criteria (National Center for Emerging and Zoonotic Infectious Disease, 2019). As directed, a county may be designated as "established" for a tick species when ≥ six ticks of a single life stage or > one life stage of a single species are collected from the county within 12 months. A county may be designated as "reported" for a tick

species when < six ticks of a single life stage are collected from the county within a 12-month period. Since there is greater confidence in the presence of a species rather than the absence of a species, once a county is classified as “established” it will not revert to a ”reported” or “no records” status. Similarly, once a county is classified as “reported” it will not revert to a “no records” status.

2.4 Results

2.4.1 Summary

I collected 674 *I. scapularis* ticks (558 adults, 60 nymphs, 56 larvae) from 58 counties in Kentucky between March 2019 and December 2022 (Table 2.1). *Borrelia burgdorferi* was detected in *I. scapularis* from 16 counties (Figure 2.7). *Ixodes scapularis* was collected every month of the year, though this differed by year and between field collections and submissions (Figures 2.4, 2.5, & 2.6). I characterized 23 established counties and 36 reported counties during the four-year study period (Figure 2.8).

2.4.2 Active surveillance

A total of 200 (29.7% of all ticks in the study) *I. scapularis* ticks (95 adults, 50 nymphs, and 55 larvae) were collected from the field (Table 2.2). During the study period, 106 counties were dragged for ticks, and I collected *I. scapularis* from the field in 38 of those counties (Table 2.4). Ticks were collected primarily from predominately forested sites (48.9%) but were also collected in mixed forest and brush (19.1%), mixed forest and grassland (10.6%), mixed grassland and brush (6.4%), and in predominate grassland habitats (2.1%). Collections occurred during temperatures between 48° and 91° Fahrenheit. I collected ticks in the field every month except for February, September, and December.

From my field collections, peak activity was seen in November for adults, June for nymphs, and July for larvae (Figure 2.5).

2.4.3 Passive surveillance

A total of 474 (70.3% of all ticks in the study) *I. scapularis* ticks (463 adults, ten nymphs, and one larva) were received by submission through the program (Table 2.3). Out of the 1,107 submissions received between 2019-2022, 168 submissions (15.2% of all submissions) contained *I. scapularis* ticks. Ticks were submitted from dog, cat, human, bird, and deer hosts. A small number of submissions failed to include the host type and species; therefore, the host type was classified as an "undetermined animal". I accepted submissions from all 120 of Kentucky's counties and received submissions containing *I. scapularis* from 36 counties (Table 2.4). This species was collected and submitted every month except for July. Adults were received in the highest abundance in November, and nymphs and the single larva were received in the highest abundance in May (Figure 2.6).

2.4.4 Detection of *B. burgdorferi*

Borrelia burgdorferi was detected in field-collected ticks from seven counties and in submitted ticks from 12 counties (Table 2.4). The minimum infection rate (MIR) was calculated as the ratio of the number of positive pools to the total number of specimens tested assuming each positive pool contained one infected tick. The overall MIR for *B. burgdorferi* in this study was 4.46%. Adults had a MIR of 4.63% and nymphs had a MIR of 3.77%. Per the standards noted in the CDC's guidelines for prostriate tick surveillance, engorged ticks were not included in this prevalence calculation (CDC, 2020).

2.4.5 County establishment status

I made progress establishing Kentucky counties' statuses every year of the study. Out of the 58 counties where *I. scapularis* was collected, 23 were classified as established counties and 35 as reported counties. In 2019, six counties were classified as established and 11 counties as reported. An additional six counties were classified as established and an additional 10 counties as reported in 2020. An additional six counties as established and an additional 12 counties as reported in 2021, and then an additional five counties as established, and an additional nine counties as reported in 2022. Sixty counties were classified as with "no records" due to the lack of collection of *I. scapularis* from those counties (Figure 2.8).

2.5 Discussion

Historically, *I. scapularis* distribution in the Southeast is infrequent, and infection by *B. burgdorferi* uncommon, leading to the perception that Lyme disease rarely occurred outside of the northeastern United States. Increases in human Lyme disease cases have challenged this idea and led to many states performing surveillance and finding both the tick and pathogen in varied abundance. In this study, the main objective was to identify the distribution of *I. scapularis* and *B. burgdorferi* in Kentucky, but I also collected data on seasonality, habitat, population status, and estimated prevalence of infection.

Most collections came from a forest or forest-mixed environment (78.6%). Forest ecosystem characteristics such as canopy cover, humidity, and plentiful leaf litter contribute to the establishment of *I. scapularis* as they have limited desiccation tolerance and require dense, humid microenvironments to survive (Gardner et al., 2020, Ginsberg et al., 2020). The majority of counties where I identified *I. scapularis* are in the eastern half

of Kentucky, which is dominated by the deciduous forests of the Appalachian Mountains—a habitat that would be most favorable to this species. Conversely, as one moves westward, elevation decreases, canopy cover decreases, and land type transitions from dense forest to open cropland—altogether, a less suitable locale for *I. scapularis.*

I identified *I. scapularis* in 58 counties. According to the CDC database for *I. scapularis* surveillance in the United States, 33 Kentucky counties are established, 20 counties are reported, and 67 counties have no record of collection as of 2021 (Centers for Disease Control and Prevention a, 2022). From this study, I report data supporting an additional 15 established and 20 reported counties. The few previous studies documenting *I. scapularis* in Kentucky report populations across the state (Dennis et al., 1998; Eisen et al., 2016, Lockwood et al., 2018), though almost all counties reported in Eisen et al., 2016 include unpublished data. I report populations in 30 counties that have not been previously identified in the *I. scapularis* range, contributing greatly to the statewide distribution of this species in Kentucky.

I incorporated methods of both active and passive surveillance in this study. Active surveillance for tick populations has shown to have high specificity, implying that the collection of several *I. scapularis* specimen from a site indicates a self-sustaining, reproducing population at that location as host-seeking ticks provide a more precise spatial distribution compared to ticks collected from hosts (Eisen & Eisen, 2018; Fleshman et al., 2021). In contrast, passive surveillance efforts are used primarily for classifying county status, distribution, and seasonality but hold limitations in determining the prevalence of pathogens in tick populations.

Over a third (36.8%) of all field-collected adult *I. scapularis* were collected in November. The passive surveillance also supports this month as a high activity time for adults, with 41.3% of all submitted adults reportedly collected in November. *Ixodes scapularis* adults in Kentucky appear to emerge in October and remain active through June. Overall, 82.9% of all adults and 70.3% of ticks collected in the study were collected through the submission program, exemplifying how effective passive efforts in the surveillance of this species can be.

Nymphs emerged in May every year of the study and continued activity until September at the latest—though I did not collect nymphs in any high abundance. Larvae were scarcely collected aside from one sampling event in July 2019 when 82.1% of all larvae were collected. The small number of immature *I. scapularis* collected in this study, particularly nymphs, is a limitation of this study. However, I can conclude that this species appears active year-round, though exposure to adults is more likely than that of nymphs or larvae.

The lack of nymphal collection in this study may be explained by behavior. The practice of dragging for ticks is a widely accepted collection method in surveillance and is performed by dragging a sheet along the ground to allow questing ticks to latch on. The drag is checked periodically, and ticks are easily removed and collected. Observation studies of questing (i.e., host-seeking) *I. scapularis* find behavioral variability between populations in the Northeast and Southeast (Tietjen et al., 2020; Ginsberg et al., 2021). Northern populations of *I. scapularis* remain above leaf litter while host-seeking, frequently climbing onto the edges of grass and bush (Arsnoe et al., 2019). They also regularly feed on small mammals scurrying atop the litter like the white-footed mouse

(*Peromyscus leucopus*), a principal reservoir for *B. burgdorferi* in North America (Salkeld et al., 2008). This behavior permits better access to human hosts, an increased chance of infection by *B. burgdorferi*, and brands this population as more "draggable". In contrast, southern populations linger closer to the ground beneath leaf litter—ultimately isolated from anything that remains above the leaf litter barrier—and attach to reptiles which act as poor reservoirs for the bacteria (Apperson et al., 1993; Durden et al., 2002). The differences in the behavior of these two populations impact the frequency of utilizing humans as hosts, infection by the Lyme disease bacterium, and collection frequency via drag. Nymphs pose the highest risk of transmission to humans largely because their small size allows them to inconspicuously hide and feed on hosts (Fish, 1993 & 1995). Therefore, additional surveillance utilizing methods targeted at collecting southern *I. scapularis* nymphs (i.e., host-trapping) could provide more data. However, it should be noted that the genetics of *I. scapularis* in Kentucky have not been investigated, so it is unclear which population, northern or southern, is here.

I detected *B. burgdorferi* in *I. scapularis* from 16 counties and calculated an overall MIR of 4.5%. The minimal infection rate is calculated as the ratio of the number of positive pools to the total number of specimens in the sample. In Kentucky, the only other studies investigating *B. burgdorferi* in *I. scapularis* report detections in Bell, Boyd, Casey, Greenup, Hart, Henry, Jackson, Lyon, and Russell counties (Taft et al., 2005; Lockwood et al., 2018) but no prevalence is reported. The analysis of over 2,000 records compiled from literature, publicly available tick-borne pathogen surveillance databases, and internal CDC pathogen databases in Fleshman et al., 2021 found no counties in Kentucky with *B.*

burgdorferi in host-seeking *I. scapularis*. In this study, I found *B. burgdorferi* in host-seeking *I. scapularis* collected from seven counties.

The detection of *B. burgdorferi* in Appalachia and southeastern states' blacklegged tick populations has changed in recent years, documenting a trend of *B. burgdorferi*'s development in these regions. The lack of previous studies in Kentucky makes comparisons over time difficult. Fortunately, surrounding states have done significantly more research, though the infection rates seem to vary considerably.

Previous testing for *B. burgdorferi* in southwestern Virginia found infection among 33% of collected host-seeking *I. scapularis* (Herrin et al., 2014). Prior studies had documented infection in *Ixodes* populations along the coastline of Virginia but reports of infected populations in more inland regions of the state were unavailable (Nadolny et al., 2011). A publication reporting data collected in 2007-2008 in Tennessee failed to detect any *Borrelia spp.* in >800 *I. scapularis*, and a following survey also found no Lyme spirochetes in the collected samples (Mays et al., 2014; Rosen et al., 2014). Then, Hickling et al., 2018 detected *B. burgdorferi* in 9.6% of *I. scapularis* collected from the upper Tennessee Valley and identified two sites of high infection where the detection rate was 44% and 78% in Union County, TN. In the same year, Lockwood et al., 2018 noted that *B. burgdorferi* was found in *I. scapularis* at numerous sites across Kentucky in their study (though no prevalence was reported). The reasons for these discrepancies are up for debate, as some hypothesize that lower infection rates of reservoir hosts are to blame, while others suggest more complex factors that influence the survivability of *B. burgdorferi* in the tick (Roome et al., 2017; Arsnoe et al., 2019; Ginsberg et al., 2021). Since data on this topic in Kentucky is scarce, it is difficult to make comparisons, though the infection rate in this

study appears to fall within the confines of what is estimated in neighboring states. Nonetheless, this study, in addition to the few others, should serve as a reference upon which additional studies can compare findings and guide future tick and tick-borne pathogen surveillance efforts in Kentucky.

Limitations of this study are recognized as follows. 1) It was not feasible for us to actively survey for ticks in each of Kentucky's 120 counties, so much of the data is reliant upon the information provided in the submission program. 2) I was also not able to perform repeated collections over the study period in the same sample sites. From this, variable sample sizes and insufficient ticks in some counties, which may have led to the lack of detection in those counties, are major limitations. Further, the presence of northern or southern *I. scapularis* populations, as discussed, may also have impacted my ability to collect this species as nymphs. 3) Most of the *I. scapularis* collected in this study were received through the submission program. While this type of data is useful in determining the presence/absence of pathogens in the collected ticks, it cannot be used in pathogen prevalence calculations (such as MIR) since there is no way to distinguish whether the infection was present in the tick or in the bloodmeal of its host.

The distribution of *I. scapularis* in the United States has been a popular topic of surveillance for several years. As the range of this vector continues to expand, it remains vital that surveillance efforts are ongoing to understand this expansion and monitor the coexisting expansion of Lyme disease. While this study presents an updated distribution of the presence of *B. burgdorferi* and *I. scapularis* populations, additional studies are needed. I identified *I. scapularis* from 58 Kentucky counties—30 that have had no previous reports of this tick—and detected the Lyme disease spirochete, *B. burgdorferi*, in 16 of these

counties, adding to the few previous reports of *I. scapularis* and *B. burgdorferi* in Kentucky. As reports of human and animal Lyme disease cases increase, I highlight the paralleled need for more surveillance in Kentucky to ensure that state data is current and accurate. As exposure to ticks is the primary risk factor for developing any tick-borne illness, outreach efforts to communicate personal protective measures such as avoiding tick habitat, wearing long pants, and appropriate use of vector repellents must be communicated.

Table 2.1 Yearly collections of *I. scapularis* by life stage and month.

2019													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	14	0	2	1	0	0	0	5	9	0	31
NYMPHS	0	0	0	0	3	1	7	0	0	0	0	0	11
LARVAE	0	0	0	0	1	1	46	1	0	0	0	0	49
Ticks collected per month	0	0	14	0	6	3	53	1	0	5	9	0	91
2020													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	6	0	3	0	8	0	0	0	0	8	161	157	343
NYMPHS	0	0	0	0	4	4	7	1	0	0	0	0	16
LARVAE	0	0	0	0	0	0	5	2	0	0	0	0	7
Ticks collected per month	6	0	3	0	12	4	12	3	0	8	161	157	366
2021													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	1	17	13	6	2	0	0	0	2	38	1	80
NYMPHS	0	0	0	0	3	11	1	0	0	0	0	0	15
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	0	1	17	13	9	13	1	0	0	2	38	1	95
2022													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	16	1	28	16	2	0	0	0	0	15	18	8	104
NYMPHS	0	0	0	0	7	7	2	1	1	0	0	0	18
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	8

Table 2.1 (continued)

Ticks collected per month	16	1	28	16	9	7	2	1	1	15	18	8	122
2019-2022													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	22	2	62	29	18	3	0	0	0	30	226	166	558
NYMPHS	0	0	0	0	17	23	17	2	1	0	0	0	60
LARVAE	0	0	0	0	1	1	51	3	0	0	0	0	56
Ticks collected per month	22	2	62	29	36	27	68	5	1	30	226	166	674

Table 2.2 Yearly field collections of *I. scapularis* by life stage and month.

2019													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	14	0	1	0	0	0	0	5	1	0	21
NYMPHS	0	0	0	0	1	0	7	0	0	0	0	0	8
LARVAE	0	0	0	0	0	1	46	1	0	0	0	0	48
Ticks collected per month	0	0	14	0	2	1	53	1	0	5	1	0	77
2020													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	1	0	0	0	8	0	0	0	0	0	16	0	25
NYMPHS	0	0	0	0	3	4	7	0	0	0	0	0	14
LARVAE	0	0	0	0	0	0	5	2	0	0	0	0	7
Ticks collected per month	1	0	0	0	11	4	12	2	0	0	16	0	46
2021													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	1	0	3	1	0	0	0	0	15	0	20
NYMPHS	0	0	0	0	3	11	1	0	0	0	0	0	15
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	0	0	1	0	6	12	1	0	0	0	15	0	35
2022													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	15	10	0	0	0	0	0	1	3	0	29
NYMPHS	0	0	0	0	4	7	2	0	0	0	0	0	13
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2.2 (continued)

Ticks collected per month	0	0	15	10	4	7	2	0	0	1	3	0	42
2019-2022													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	1	0	30	10	12	1	0	0	0	6	35	0	95
NYMPHS	0	0	0	0	11	22	17	0	0	0	0	0	50
LARVAE	0	0	0	0	0	1	51	3	0	0	0	0	55
Ticks collected per month	1	0	30	10	23	24	68	3	0	6	35	0	200

Table 2.3 Yearly collections of submitted *I. scapularis* by life stage and month.

2019													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	0	1	1	0	0	0	0	8	0	10
NYMPHS	0	0	0	0	2	1	0	0	0	0	0	0	3
LARVAE	0	0	0	0	1	0	0	0	0	0	0	0	1
Ticks collected per month	0	0	0	0	4	2	0	0	0	0	8	0	14
2020													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	5	0	3	0	0	0	0	0	0	8	145	157	318
NYMPHS	0	0	0	0	1	0	0	1	0	0	0	0	2
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	5	0	3	0	1	0	0	1	0	8	145	157	320
2021													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	1	16	13	3	1	0	0	0	2	23	1	60
NYMPHS	0	0	0	0	0	0	0	0	0	0	0	0	0
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	0	1	16	13	3	1	0	0	0	2	23	1	60
2022													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	16	1	13	6	2	0	0	0	0	14	15	8	75
NYMPHS	0	0	0	0	3	0	0	1	1	0	0	0	5
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2.3 (continued)

Ticks collected per month	16	1	13	6	5	0	0	1	1	14	15	8	80
2019-2022													
Life Stage of *I. scapularis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	21	2	32	19	6	2	0	0	0	24	191	166	463
NYMPHS	0	0	0	0	6	1	0	2	1	0	0	0	10
LARVAE	0	0	0	0	1	0	0	0	0	0	0	0	1
Ticks collected per month	21	2	32	19	13	3	0	2	1	24	191	166	474

Table 2.4 Table listing counties where *I. scapularis* was collected from the field vs. from submission.

	Collected from the field		Collected via submission	
Kentucky County List	*I. scapularis* collected	*B. burgdorferi* detected	*I. scapularis* collected	*B. burgdorferi* detected
Adair County	X			
Allen County				
Anderson County				
Ballard County				
Barren County				
Bath County				
Bell County				
Boone County			X	X
Bourbon County				
Boyd County			X	
Boyle County	X		X	X
Bracken County				
Breathitt County	X			
Breckinridge County			X	
Bullitt County	X			
Butler County				
Caldwell County				
Calloway County				
Campbell County	X		X	X
Carlisle County				
Carroll County				
Carter County			X	
Casey County			X	
Christian County	X	X		

Table 2.4 (continued)

Clark County				
Clay County				
Clinton County				
Crittenden County				
Cumberland County	X			
Daviess County			X	
Edmonson County	X			
Elliott County				
Estill County			X	
Fayette County	X		X	X
Fleming County			X	
Floyd County			X	
Franklin County				
Fulton County				
Gallatin County	X			
Garrard County	X			
Grant County	X		X	
Graves County				
Grayson County				
Green County				
Greenup County				
Hancock County	X			
Hardin County	X			
Harlan County				
Harrison County				
Hart County				
Henderson County				
Henry County				

Table 2.4 (continued)

Hickman County				
Hopkins County				
Jackson County	X		X	X
Jefferson County	X	X	X	X
Jessamine County	X			
Johnson County				
Kenton County			X	X
Knott County			X	
Knox County			X	X
Larue County	X			
Laurel County	X	X	X	
Lawrence County				
Lee County				
Leslie County	X		X	
Letcher County				
Lewis County				
Lincoln County	X			
Livingston County				
Logan County				
Lyon County				
Madison County	X		X	X
Magoffin County				
Marion County				
Marshall County	X	X		
Martin County				
Mason County	X	X		
McCracken County			X	
McCreary County				

Table 2.4 (continued)

McLean County			X	
Meade County	X			
Menifee County				
Mercer County	X	X	X	X
Metcalfe County	X			
Monroe County				
Montgomery County				
Morgan County				
Muhlenberg County	X			
Nelson County			X	
Nicholas County				
Ohio County				
Oldham County	X			
Owen County	X			
Owsley County	X			
Pendleton County	X		X	
Perry County				
Pike County	X			
Powell County				
Pulaski County	X		X	
Robertson County				
Rockcastle County	X	X	X	X
Rowan County				
Russell County			X	
Scott County	X		X	
Shelby County			X	
Simpson County				
Spencer County			X	

Table 2.4 (continued)

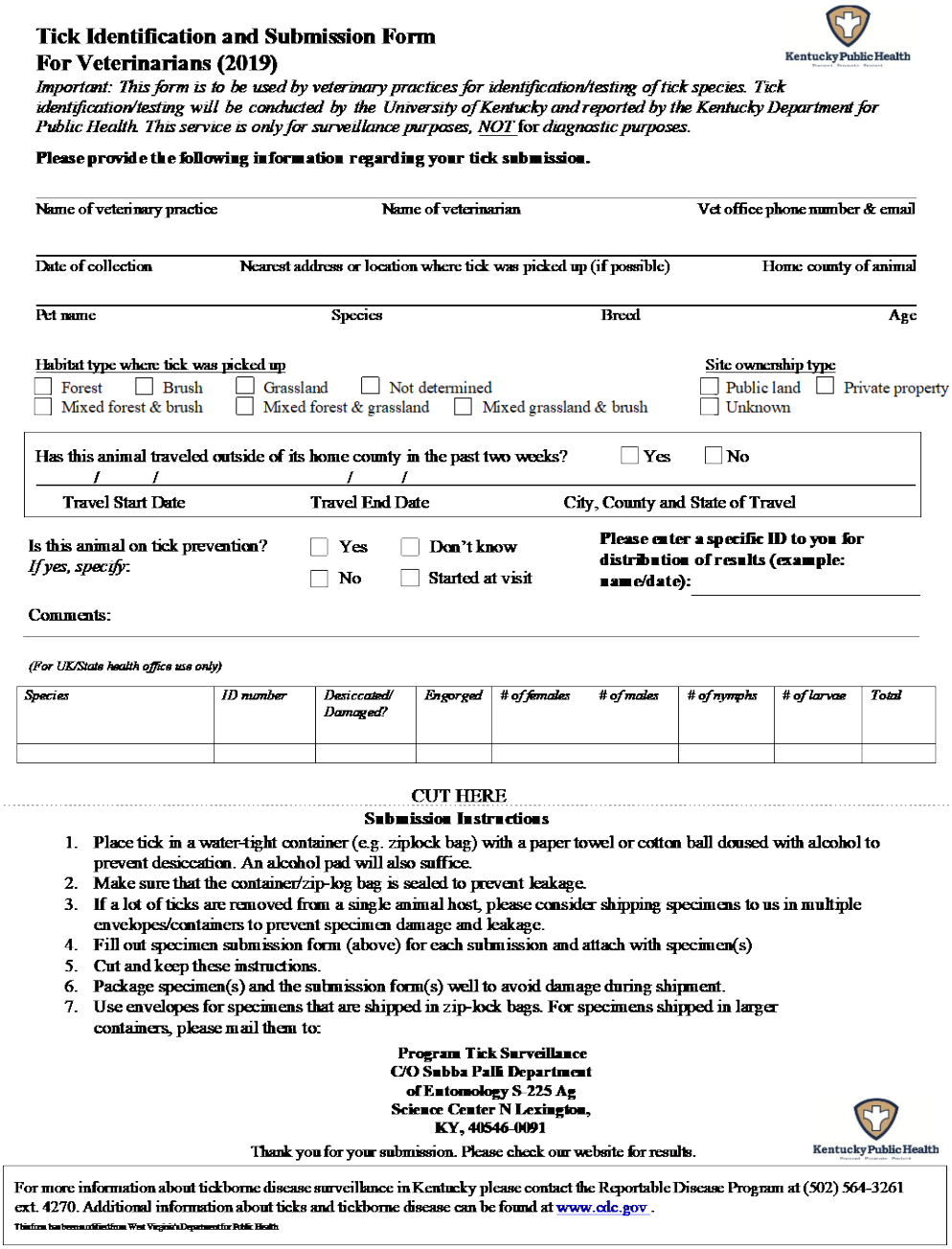
Taylor County				
Todd County				
Trigg County				
Trimble County			X	
Union County				
Warren County				
Washington County				
Wayne County				
Webster County				
Whitley County			X	
Wolfe County	X		X	
Woodford County	X		X	X

Figure 2.1 ***Ixodes scapularis*** **nymph collected on the drag-cloth.**

This method uses a one-meter by one-meter light-colored cloth attached to a dowel rod that is pulled along the ground at a slow pace by an attached rope. Questing ticks attach to the cloth as it passes by them. Ticks are collected from the cloth every ten meters using forceps and placed into collection vials.

Figure 2.2 Veterinary submission form.

The form used by veterinarians for submission of ticks through the Kentucky Veterinarian Tick Submission Program.



Tick Identification and Submission Form
For Veterinarians (2019)

Kentucky Public Health

Important: This form is to be used by veterinary practices for identification/testing of tick species. Tick identification/testing will be conducted by the University of Kentucky and reported by the Kentucky Department for Public Health. This service is only for surveillance purposes, NOT for diagnostic purposes.

Please provide the following information regarding your tick submission.

Name of veterinary practice | Name of veterinarian | Vet office phone number & email

Date of collection | Nearest address or location where tick was picked up (if possible) | Home county of animal

Pet name | Species | Breed | Age

Habitat type where tick was picked up
☐ Forest ☐ Brush ☐ Grassland ☐ Not determined
☐ Mixed forest & brush ☐ Mixed forest & grassland ☐ Mixed grassland & brush

Site ownership type
☐ Public land ☐ Private property
☐ Unknown

Has this animal traveled outside of its home county in the past two weeks? ☐ Yes ☐ No

Travel Start Date (__/__/__) | Travel End Date (__/__/__) | City, County and State of Travel

Is this animal on tick prevention? ☐ Yes ☐ Don't know ☐ No ☐ Started at visit
If yes, specify:

Please enter a specific ID to you for distribution of results (example: name/date): _____

Comments:

(For UK/State health office use only)

Species	*ID number*	*Desiccated/ Damaged?*	*Engorged*	*# of females*	*# of males*	*# of nymphs*	*# of larvae*	*Total*

CUT HERE

Submission Instructions

1. Place tick in a water-tight container (e.g. ziplock bag) with a paper towel or cotton ball doused with alcohol to prevent desiccation. An alcohol pad will also suffice.
2. Make sure that the container/zip-log bag is sealed to prevent leakage.
3. If a lot of ticks are removed from a single animal host, please consider shipping specimens to us in multiple envelopes/containers to prevent specimen damage and leakage.
4. Fill out specimen submission form (above) for each submission and attach with specimen(s)
5. Cut and keep these instructions.
6. Package specimen(s) and the submission form(s) well to avoid damage during shipment.
7. Use envelopes for specimens that are shipped in zip-lock bags. For specimens shipped in larger containers, please mail them to:

Program Tick Surveillance
C/O Subba Palli Department
of Entomology S-225 Ag
Science Center N Lexington,
KY, 40546-0091

Thank you for your submission. Please check our website for results.

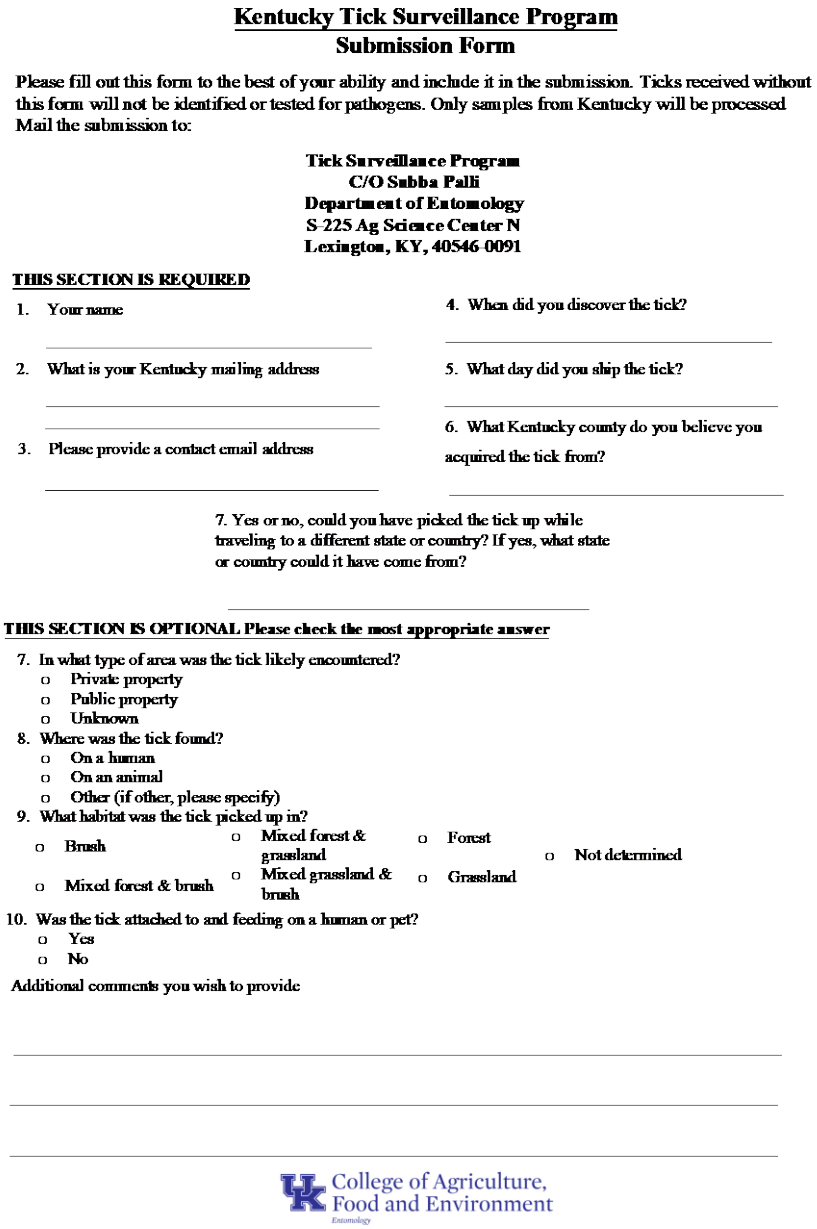
Kentucky Public Health

For more information about tickborne disease surveillance in Kentucky please contact the Reportable Disease Program at (502) 564-3261 ext. 4270. Additional information about ticks and tickborne disease can be found at www.cdc.gov .

This form has been modified from West Virginia's Department for Public Health

Figure 2.3 Non-veterinary submission form.

The form, used by non-veterinarians for the submission of ticks through the Kentucky Tick Submission Program.



Kentucky Tick Surveillance Program
Submission Form

Please fill out this form to the best of your ability and include it in the submission. Ticks received without this form will not be identified or tested for pathogens. Only samples from Kentucky will be processed
Mail the submission to:

Tick Surveillance Program
C/O Subba Palli
Department of Entomology
S-225 Ag Science Center N
Lexington, KY, 40546-0091

THIS SECTION IS REQUIRED

1. Your name _____
2. What is your Kentucky mailing address _____
3. Please provide a contact email address _____
4. When did you discover the tick? _____
5. What day did you ship the tick? _____
6. What Kentucky county do you believe you acquired the tick from? _____
7. Yes or no, could you have picked the tick up while traveling to a different state or country? If yes, what state or country could it have come from? _____

THIS SECTION IS OPTIONAL Please check the most appropriate answer

7. In what type of area was the tick likely encountered?
   - o Private property
   - o Public property
   - o Unknown
8. Where was the tick found?
   - o On a human
   - o On an animal
   - o Other (if other, please specify)
9. What habitat was the tick picked up in?
   - o Brush
   - o Mixed forest & grassland
   - o Forest
   - o Not determined
   - o Mixed forest & brush
   - o Mixed grassland & brush
   - o Grassland
10. Was the tick attached to and feeding on a human or pet?
    - o Yes
    - o No

Additional comments you wish to provide

_____

_____

_____

College of Agriculture, Food and Environment
Entomology

Figure 2.4 ***Ixodes scapularis*** **collections per month and life stage for all years of the study.**

Bar chart shows the number of *I. scapularis* collected in total per month for all years of the study.

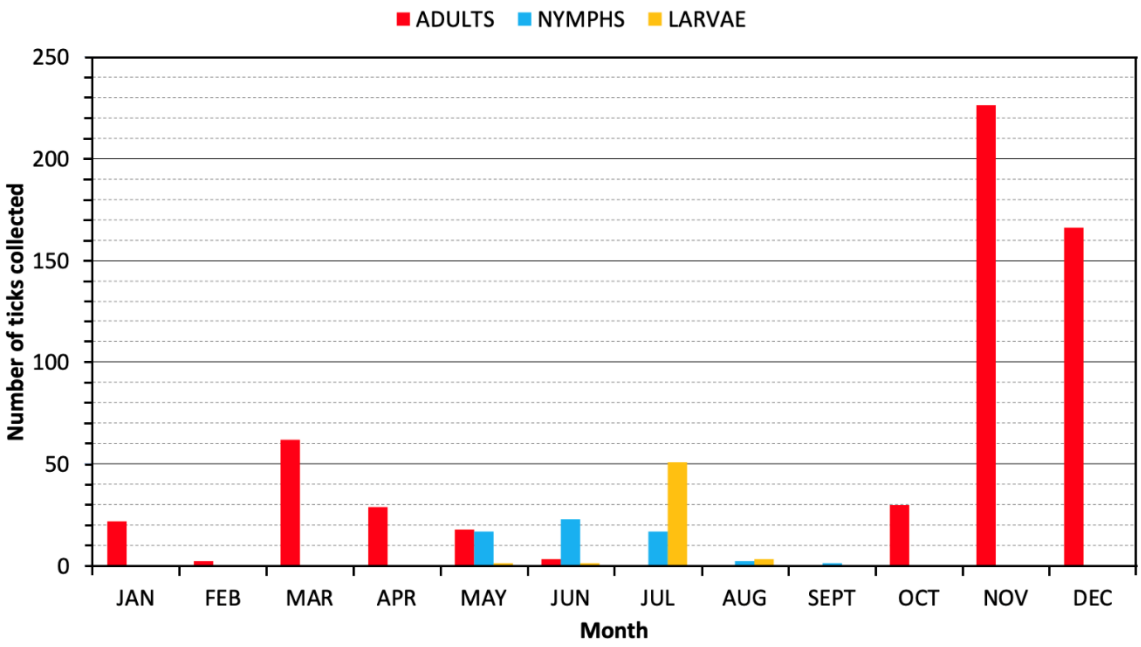


Figure 2.5 *Ixodes scapularis* field collections per month and life stage for all years of the study.

Bar chart shows the number of field-collected *I. scapularis* collected in total per month for all years of the study.

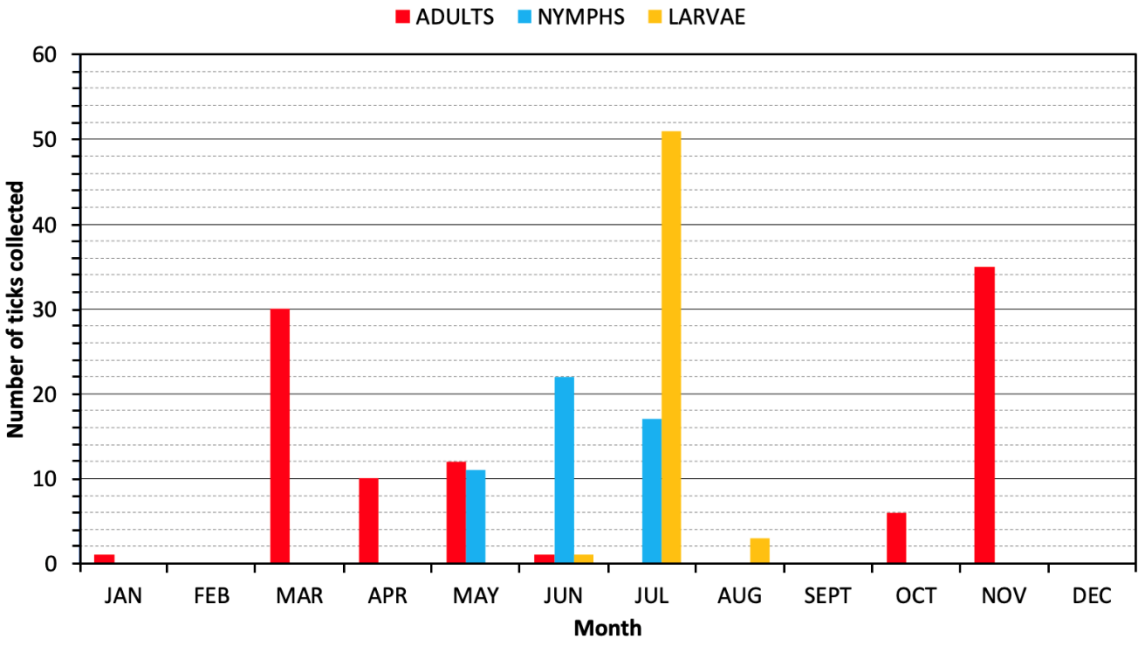


Figure 2.6 *Ixodes scapularis* submitted collections per month and life stage for all years of the study.

Bar chart shows the number of submitted *I. scapularis* collected in total per month for all years of the study.

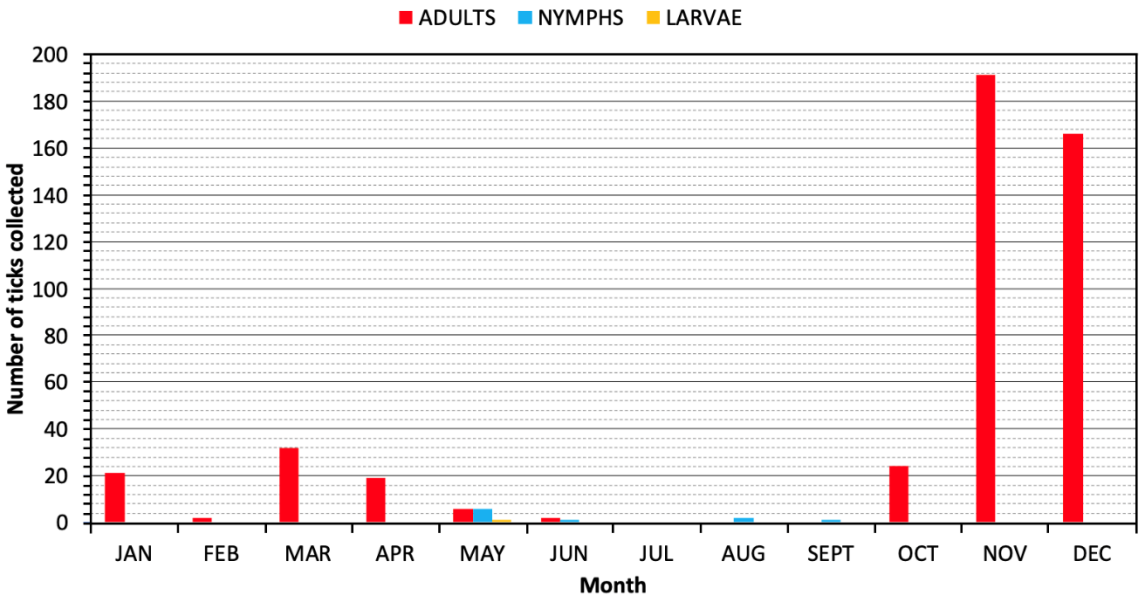


Figure 2.7 County-level distribution of *I. scapularis* and *B. burgdorferi* in Kentucky. Counties shaded grey represent counties where *I. scapularis* ticks were collected, either in the field or through submission. Counties with a black dot represent detection of *B. burgdorferi* in only field-collected ticks from that county, a white dot represents detection in only submitted ticks from that county, and a half-black half-white circle represents detection in both field-collected and submitted ticks from that county.

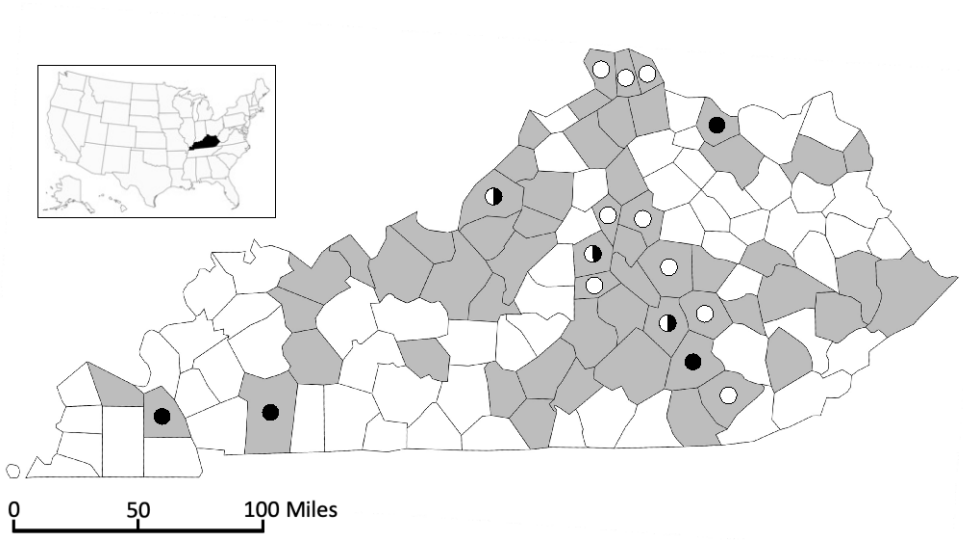


Figure 2.8 County establishment status for *I. scapularis* each year of this study.

Per guidelines, once a county is classified as "established" it will not revert to a "reported" or "no records" status, and once a county is classified as "reported" it will not revert to a "no records" status.

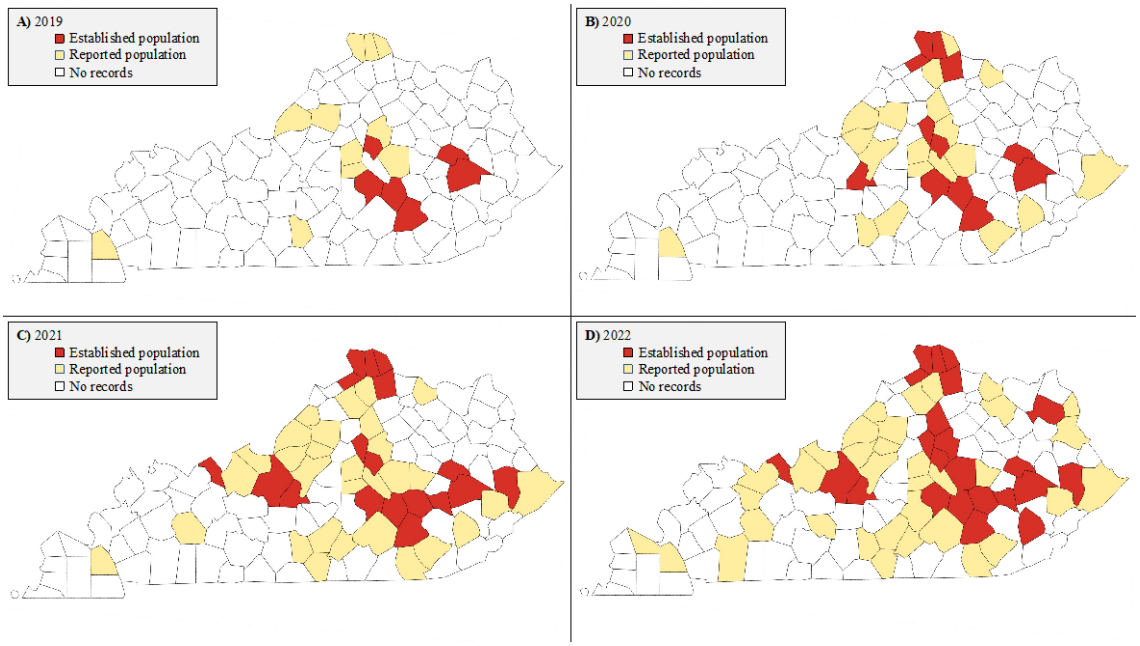
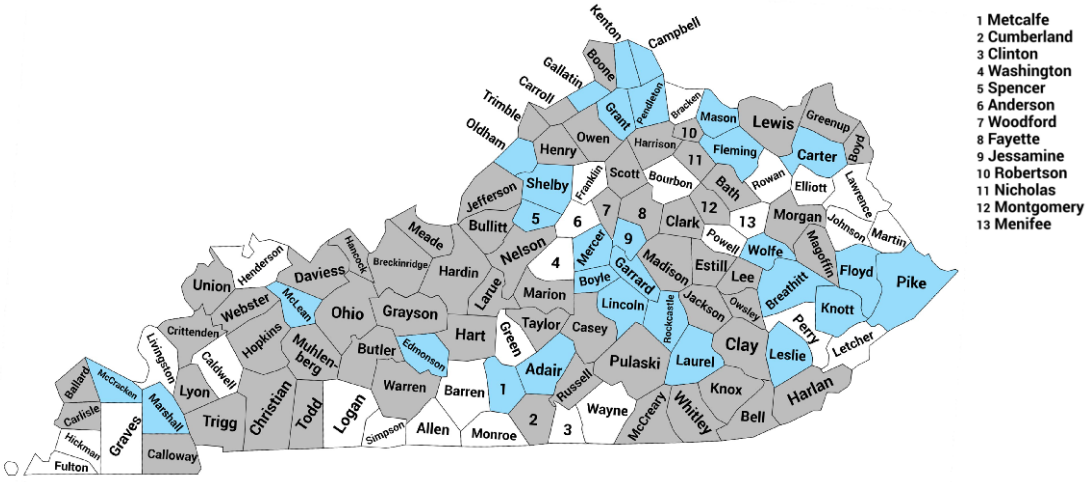


Figure 2.9 Updated distribution of *I. scapularis* compared to other sources.

The distribution of *I. scapularis* previously reported in either Dennis et al., 1998; Eisen et al., 2016, Lockwood et al., 2018, or the current ArboNET database. Counties in blue represent counties with *I. scapularis* populations identified in this study, but otherwise have no prior reports of this species.



CHAPTER 3. COUNTY-LEVEL SURVEILLANCE FOR THE LONE STAR TICK, *AMBLYOMMA AMERICANUM* AND ITS ASSOCIATED PATHOGEN, *EHRLICHIA CHAFFEENSIS*, IN KENTUCKY

This chapter contains data published in an academic journal (Pasternak & Palli, 2023) but has been updated to include data from the years 2021 and 2022 that have not been published.

3.1 Abstract

Kentucky is among the states with the highest incidence rates for ehrlichiosis, a bacterial infection caused by the pathogen *Ehrlichia chaffeensis* and transmitted to humans primarily by *Amblyomma americanum*, commonly known as the lone star tick. *Amblyomma americanum* is common to Kentucky and much of the southeast but has expanded farther north in recent years. As an abundant and aggressive nondiscriminatory biter, this species is a major public health concern for transmitting pathogens to humans. With this tick's range expanding northward, it poses a significant public health concern, making surveillance critical to tracking its expansion over time. However, historical data on tick distribution in Kentucky is limited. To address this, I conducted surveillance for *A. americanum* in Kentucky from January 2019 to December 2022, through field collections and a statewide tick submission program established in collaboration with the Kentucky Department for Public Health. I screened for *E. chaffeensis* on a county level throughout the state and collected 8,047 *A. americanum* ticks from 115 counties, detecting *E. chaffeensis* in 44 counties. The minimum infection rate was 2.2%. With the expanding range of *A. americanum* and increasing cases of tick-borne diseases, ongoing surveillance

is necessary to monitor this important tick vector and track the spread of tick-borne illnesses over time.

KEYWORDS: ticks, vector surveillance, *Amblyomma americanum*, *Ehrlichia chaffeensis*, Kentucky.

3.2 Introduction

Amblyomma americanum, commonly known as the lone star tick, is a three-host tick found throughout the southeastern United States. This species is of major concern as a vector of several pathogens causing disease in both animals and humans, particularly ehrlichiosis (Goddard et al 2009; Eisen et al 2017), and is the most common tick to bite humans in the southeastern United States due to their aggressive and unbiased biting habits (Stromdahl and Hickling 2012; Gaines et al., 2014). Well-established throughout the southeastern U.S., it is expanding its range northward into the northeastern and northern Midwest of the United States, and further into Canada (Molaei et al 2019; Nelder et al 2019). In Kentucky, this species has been documented in studies prior to the 21st century (Schreck et al 1980; Mount & Snoddy 1983; Goddard & Norment 1986; Bloemer et al 1990) with more detailed descriptions of county-level presence in later publications (Fritzen et al 2011; Lockwood et al 2018; Slabach et al. 2018). However, there is still a lack of information on the distribution of this medically & economically important vector statewide.

While Lyme disease remains the most commonly reported tick-borne disease in the United States and is particularly prevalent in the northeast and northern Midwest, it is not as frequent amongst the southeast where, instead, Rickettsial diseases are more often reported. Rickettsial illnesses are caused by a variety of bacteria from the order Rickettsiales and genera *Rickettsia, Anaplasma, Ehrlichia*, *NeoRickettsia, NeoEhrlichia*, and *Orientia* (Nicholson et al 2019). Ehrlichiosis in the United States is primarily caused by *Ehrlichia chaffeensis* but may also result from infection by *E. ewingii* or *E. muris eauchairensis*. *Ehrlichia chaffeensis* and *E. ewingii* are predominantly vectored by *A.*

americanum while the third bacterium, *E. muris eauclairensis*, is vectored by *Ixodes scapularis* (Pritt et al. 2011).

In 2011, the Center for Disease Control and Prevention (CDC) reported 863 human cases of ehrlichiosis in the United States. Over the next seven years, cases steadily increased and in 2018, 1,832 human cases were reported—1,799 of which were caused by *E. chaffeensis* infection (Adams et al 2013). *Ehrlichia chaffeensis* is part of the family *Anaplasmataceae* and persists in nature by cycling between vector and host, such as the white-tailed deer (*Odocoileus virginianus*) which is widespread in Kentucky (Zimmerman et al 1988; Felz et al 1996; Lockhart et al 1997; Yabsley et al 2005; Yabsley 2010). When *E. chaffeensis* infects humans, a dead-end host, illness occurs as the bacteria infect blood leukocytes and cause low levels of white blood cells and platelets. While often mild, it can progress and be fatal if left untreated or in those with compromised immune systems. Symptoms of ehrlichiosis are like those of other tick-borne illnesses (ex., fever, aches, and chills) while 30% of infected adults and 60% of infected children develop a spotted rash (Paddock & Childs, 2003).

Issues of standardizing tick surveillance and underreporting of tick-borne disease limit understanding of tick-borne disease prevalence and spread in the United States. As ticks often expand to new areas from pressures of climate change and transportation by hosts, their ranges remain everchanging. Previous surveillance studies have reported on the presence of *A. americanum* in parts of Kentucky, however, there is still a lack of published data on the distribution of this tick statewide. As prevention of tick-borne diseases relies significantly on an accurate understanding by public health officials and healthcare

providers of when and where persons are at risk for exposure to ticks and to the pathogens they transmit, surveillance remains fundamental to “fight the bite”.

Here, I report on my findings of *A. americanum* and *E. chaffeensis* distribution in Kentucky counties from 2019 to 2022. The objectives were to 1) determine where *A. americanum* populations were present and 2) if *E. chaffeensis* was present in those tick populations. Because Kentucky has 120 counties, it was not practical for us to perform active surveillance in every county. Therefore, I utilized passive surveillance through the form of a tick submission program so that I could attempt the collection of data from as many Kentucky counties as possible. Since my study objective was determining the presence/absence of both tick and pathogen, using these two methods together was sufficient, as outlined in the CDC’s Guide to the Surveillance of Metastriate Ticks (Acari: Ixodidae) and their Pathogens in the United States (National Center for Emerging and Zoonotic Infectious Disease, 2020).

3.3 Materials and Methods

3.3.1 Active surveillance

Data collection occurred from January 2019 to December 2022. Ticks were collected from the environment by dragging. The drag cloth consisted of a one-meter by one-meter light-colored cloth attached to a dowel rod that was pulled along the ground at a slow pace by an attached rope (Figure 3.1). Sampling sites where ticks were collected included hiking trails, wildlife management areas, and other publicly accessible recreation areas. If an appropriate public site could not be accessed, collections occurred on private land with the owner's permission. All ticks were removed from drags and collected in vials

containing 70% ethanol for transport back to the laboratory at the University of Kentucky and stored at -20ºC until identification.

3.3.2 Passive surveillance

Veterinary practices in all 120 of Kentucky's counties were invited to submit ticks removed from animals brought into their clinic between March 2019 through December 2022 as part of the Kentucky Veterinarian Tick Submission Program. Participants were provided instructions on how to package and ship the tick for submission and filled out a form to collect basic contact information as well as the date of collection, county of collection, host species, age, and breed, travel outside the home county in the two weeks preceding, the use of tick preventative medicine (if applicable), habitat type in which the tick was collected and the site ownership type. All specimens were shipped to the University of Kentucky Department of Entomology.

In addition, I accepted ticks from non-veterinary practices, including health departments, physician offices, and the public. With each submission, information forms were included to obtain basic contact information, the date the tick was collected and shipped, county of collection, travel outside the home county in the two weeks preceding, habitat type in which the tick was believed to be collected, site ownership type, host type, and whether the tick was attached or not. Directions on packaging and shipping the tick were also included. Samples from outside the state of Kentucky or instances when the date and/or county-level location information could not be provided were not accepted. All ticks received through these submission programs were stored under the same conditions as ticks collected from the environment.

3.3.3 Tick identification

Ticks were identified and sexed morphologically by comparison with standard keys in the laboratory at the University of Kentucky (Keirans et al. 1989; Egizi et al. 2019). Ticks that were identified as anything other than the target organism, *A. americanum*, were omitted from this study and included in other surveillance reports (Pasternak & Palli, 2022).

3.3.4 DNA Extraction

Ticks were screened for the presence of *E. chaffeensis* using qPCR. For DNA extraction, individual ticks were bead-beaten with 2.0 mm Zirconia beads from BioSpec Products in a Tissueminser (MP Biomedicals) at a speed of 0.6 m/s for three consecutive cycles of 40 s each to ensure proper lysis. The homogenate for each tick in the sample was then pooled. DNA extraction was performed using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturer's instructions. Since the objective was to determine the presence/absence of the pathogens, I pooled ticks for testing. Ticks were not combined from multiple submissions/collection events, hosts, or locations for testing. Individual tick testing was not performed. When pooling ticks, no more than 5 adults were used per sample. Nymphs were pooled regardless of number. The remaining homogenate was stored at -20ºC for future study.

3.3.5 Detection of *E. chaffeensis*

Ten µL PCR reactions were set up containing 5 uL SYBR Green Master Mix (Bio-Rad), 2 uL extracted DNA, 0.5 uL of forward primer, 0.5 uL of reverse primer, and 2 uL nuclease-free water. *E. chaffeensis* screening was performed with primers dsb-F (TTGGAGAAGCATCACTGAAAGC) and dsb-R

(GCAGCATGGTAGAACTCGATGTA) (Wroblewski et al. 2017). Each qPCR run included one positive control of DNA confirmed to contain *E. chaffeensis* DNA and two negative controls containing non-target DNA and no DNA (nuclease-free water). The qPCR reactions were run on the Bio-Rad iCycler at the following settings: 1 cycle of 95ºC for 5 minutes, 45 cycles of 95ºC for 15 seconds, and 60ºC for 60 seconds.

3.3.6 Determination of county establishment status

Following the Centers for Disease Control and Prevention's Guide to the Surveillance of Metastriate Ticks (Acari: Ixodidae) and their Pathogens in the United States, I classified county status based on the county-level establishment criteria (National Center for Emerging and Zoonotic Infectious Disease, 2020). As directed, a county may be designated as "established" for a tick species when ≥ six ticks of a single life stage or > one life stage of a single species are collected from the county within a 12-month period. A county may be designated as "reported" for a tick species when < six ticks of a single life stage are collected from the county within 12 months. Since there is greater confidence in the presence of a species rather than the absence of a species, once a county is classified as "established" it will not revert to a "reported" or "no records" status. Similarly, once a county is classified as "reported" it will not revert to a "no records" status.

3.4 Results

3.4.1 Summary

A total of 8,047 *A. americanum* ticks were collected from 115 counties during the study period of January 2019 to December 2022. Altogether, I collected 1,658 adults, 1,812 nymphs, and 4,577 larvae (Table 3.1). *Ehrlichia chaffeensis* was detected in *A. americanum* samples from 44 counties (Figure 3.5). *Amblyomma americanum* ticks were

collected from March to November across all years (Figure 3.2). Seasonality was slightly different between each year of the study: March to September for 2019 and 2022, March to October for 2020, and March to September, then in November for 2021. From the data collected here, I can document established populations of *A. americanum* in 99 counties and reported populations in 16 counties (Figure 3.6).

3.4.2 Active surveillance

A total of 5,941 (73.8% of all ticks in the study) *A. americanum* ticks (527 adults, 998 nymphs, and 4,416 larvae) were collected from the field (Table 3.2). During the study period, 106 counties were dragged for ticks, and I collected *A. americanum* from the field in 93 of those counties (Table 3.4). Ticks were collected primarily from forested sites (40.0%) but were also collected in grassland only (18.8%), brush and grassland mix (16.5%), brush and forest mix (12.9%), grassland and forest mix (10.6%), and brush only (1.17%) habitats. Collections occurred during temperatures between 49° and 93° Fahrenheit. Across the entire study period, I collected this species in the field as early as March and as late as November, though seasonality differed between years. From the field collections, peak activity was seen in June for adults and nymphs, and in August for larvae (Figure 3.3).

3.4.3 Passive surveillance

A total of 2,106 (26.2% of all ticks in the study) *A. americanum* ticks (1,131 adults, 814 nymphs, and 161 larvae) were received by submission through the program (Table 3.3). Out of the 1,107 submissions received between 2019-2022, 594 submissions (53.6% of all submissions) contained *A. americanum* ticks. Ticks were submitted from human, dog, cat, cow, and horse hosts. A small number of submissions failed to include the host

type and species; therefore, the host type was classified as an "undetermined animal". I accepted submissions from all 120 of Kentucky's counties and received submissions containing *A. americanum* from 96 counties (Table 3.4). This species was received from March to September with no deviation between all four years. Adults and nymphs were received in the highest abundance in May and larvae were received in the highest abundance in August (Figure 3.4).

3.4.4 Detection of *E. chaffeensis*

Ehrlichia chaffeensis was detected in field-collected ticks from 33 counties and submitted ticks from 21 counties (Table 3.4). The minimum infection rate (MIR) was calculated as the ratio of the number of positive pools to the total number of specimens tested, assuming each positive pool contained one infected tick. The overall MIR for *E. chaffeensis* in this study was 2.2%. Adults had a MIR of 4.05%. Nymphs had a MIR of 1.33%. The MIRs for 2019, 2020, 2021, and 2022 were 1.8%, 2.2%, 3.7%, and 1.5%, respectively. Per the standards noted in the CDC's guidelines for metastriate tick surveillance, engorged ticks were not included in this prevalence calculation (CDC 2020).

3.4.5 County establishment status

I made progress establishing Kentucky counties' statuses every year of the study. Of the 115 counties where I collected *A. americanum*, I was able to classify 99 as established counties and 16 as reported counties for this species. In 2019, I classified 33 counties as established and 19 counties as reported. I classified an additional 25 counties as established and an additional four counties as reported in 2020, an additional 23 counties as established and an additional five counties as reported in 2021, and then an additional 18 counties as established, and an additional six counties as reported in 2022. I classified

five counties with “no records” due to the lack of collection of *A. americanum* from those counties in this study. Those five counties are Bracken County, Carlisle County, Fulton County, Mason County, and Nicholas County (Figure 3.6).

3.5 Discussion

The objective of this study was to 1) determine where *A. americanum* populations were present in Kentucky counties and 2) if *E. chaffeensis* was present in those tick populations. Here, I collected 8,047 *A. americanum* ticks from 115 counties. The wide distribution of *A. americanum* ticks collected in this study suggests that, like many other states, Kentucky houses a robust population of this tick. Historical information on the distribution in Kentucky is inconsistent (Bishopp and Trembley 1945; Kellogg et al. 1971). However recent studies have improved the understanding of how medically relevant ticks are distributed across the state (Lockwood et al 2018; Slabach et al 2018).

While the estimated range of this tick encompasses the entire southeastern United States, collection records of *A. americanum* in recent large-scale published studies and databases report otherwise. Collection records compiled by Springer et al., 2014 between 1898 and 2012 found that more than half of the counties in Kentucky showed no official record of tick presence. Similarly, the ArboNET database—that is, the official national arboviral surveillance system—available through the Centers for Disease Control and Prevention reports only 39 established counties, 30 reported counties, and 51 counties with no records of *A. americanum* presence in Kentucky (Centers for Disease Control and Prevention b, 2022). My data found established populations in 101 counties (63 additional counties compared to the referenced database) and reported populations in 15 counties (nine additional counties compared to the referenced database), adding to the establishment

status of several counties across the state and exemplifying how data available outside of this study may not be entirely accurate.

Here I collected adults March-August, nymphs March-October, and larvae May-November suggesting that the highest risk of exposure exists in late spring to early fall. Similar seasonality has been found in neighboring states. In Tennessee, adults were collected March-August, nymphs March-September, and larvae July-October (Gerhardt et al 1998). Peak abundance in this study was May for both adults and nymphs, and August for larvae, though there were slight differences in peak abundance of adults and nymphs from field collections, where peak abundance was June, compared to the submissions, where peak abundance was May. In southeast Missouri, peak *A. americanum* abundance was greatest May-July while peak activity for nymphs was May-August, and July-September for larvae (Kollars et al 2000).

I collected the highest abundance of *A. americanum* in forested habitats (40.0%), although I collected them from all habitats. The wide range of habitats this species has been found to establish likely contributes to their success in range expansion (Semtner et al., 1971; Springer et al. 2014; Springer et al. 2015; Dahlgren et al. 2016). Combined with this tick's aggressive biting nature, the threat of exposure to their bite and subsequent disease is heightened. From my data, exposure appears highest in the late spring to early fall and may be particularly heightened in forested habitats. Since larvae are not known to transmit *E. chaffeensis* infection due to lack of transovarial transmission, risk exposure to this bacterium likely decreases as nymph and adult activity declines, pointing to late spring and summer as the highest risk period for pathogen exposure.

In this study, I incorporated active and passive surveillance methods for collecting *A. americanum.* The collection of questing ticks (i.e., field-collected) is the primary collection method applied in most tick surveillance programs, but the inclusion of passive surveillance has unique benefits that active surveillance can lack. Here, I collected data on *A. americanum* from more counties through the submission program than I could survey. Additionally, far more adults were submitted than were collected in the field. The lone star tick, in particular, is an aggressive and unbiased feeder with a reputation as a regular nuisance and the most common tick to bite humans (Stromdahl and Hickling 2012; Gaines et al., 2014). The data recorded in the submission forms provide information on when, where, and how persons are encountering this species—something that was not obtainable in field collections. On the other hand, a significant portion (73.8%) of collections came from the field. Data on questing ticks is highly important for understanding naturally occurring tick-pathogen dynamics and estimating the prevalence of infection. Additionally, data on climate factors, habitat preferences, and natural emergence times are understood from active surveillance efforts.

I detected *E. chaffeensis* in *A. americanum* ticks from 44 counties in Kentucky (Figure 3.5). Kentucky is considered a high-incidence state for ehrlichiosis and state case reports document disease across the state with the highest incidences concentrated around the Daniel Boone National Forest and Land Between the Lakes areas—heavily forested areas which may factor into why disease reports are heightened here (Kentucky Department for Public Health 2021). I detected this pathogen in ticks from counties of all regions of Kentucky, though most detections were in the eastern half of the state.

The overall MIR in this study was 2.2% with variation from year to year. Comparable minimal infection rates are reported in a previous study where *E. chaffeensis* was detected in 5.6% of *A. americanum* ticks in Kentucky (Fritzen et al 2011). In the literature, detection rates of *E. chaffeensis* appear to vary significantly across states, counties, and even within studies. One study in Virginia found an overall *E. chaffeensis* detection rate of 7.3% for adults and 3.4% for nymphs but reported a much higher rate of 24.5% in Fairfax County (Gaines et al 2014). Other studies from Virginia report little to no detection of *E. chaffeensis* (Whitlow et al 2022; Wright et al. 2014). In Tennessee, Cohen et al., 2010 found a 17.4% detection rate in Davidson County, Tennessee although the rate across all counties in the study was 2.6%. More robust studies comparing infection rates among even samples of lone star ticks from distinct regions of Kentucky would help identify areas of higher risk for exposure to *E. chaffeensis* and if these risks vary geographically.

This study was not without limitations. 1) The use of passive surveillance methods here restricts prevalence estimates to only a portion of the ticks included in this study since infection rates derived from blood-fed ticks are not representative of infection rates in host-seeking ticks. Although, since most collections occurred from the field, a significant limitation is not suspected. Additionally, engorged tick data can be difficult to use for determining population densities (i.e., the density of nymphs (DON)/density of infected nymphs (DIN) and density of adults (DOA)/density of infected adults (DIA)). 2) While I attempted to collect in as many counties as possible, it was not feasible for us to actively survey for ticks in each of Kentucky's 120 counties. 3) Further, I could also not perform repeated collections over the study period in the same sample sites. From this, variable

sample sizes and insufficient ticks in some counties, which may have led to the lack of pathogen detection in those counties, are major limitations. Future surveillance efforts should focus on performing repeated sampling in a subset of counties to provide more detailed data on seasonality, population density, and *E. chaffeensis* infection rates in Kentucky's *A. americanum* population.

In addition to ehrlichiosis, *A. americanum* is widely recognized as a vector of tularemia, Heartland virus, and Bourbon virus, and is infamously noted for its role in the development of the alpha-gal allergy. Up-to-date data representing when and where persons are at risk for tick bites and tick-borne diseases aids in prevention and diagnosis efforts. However, the lack of statewide surveillance studies in Kentucky has limited the knowledge of public health workers, healthcare providers, and others involved in the best methods for control, prevention, and treatment. Here, I provide data from a four-year, statewide surveillance study for *A. americanum* in response to this issue. In this study, I surveyed for the presence of the lone star tick, *A. americanum*, and its associated pathogen, *E. chaffeensis*, in Kentucky counties. I included methods of both active and passive surveillance. *A. americanum* was identified in 115 out of 120 counties, though the distribution suggests it likely exists but was undetected in the other five counties, and *E. chaffeensis* was detected in ticks from 44 counties. Major limitations in this study included uneven sample sizes and a lack of repeated sampling. Nonetheless, this study is the first statewide, multi-year surveillance effort for *A. americanum* and *E. chaffeensis* documented in Kentucky and provides undeniably useful data for the control and management of lone star ticks and ehrlichiosis.

Table 3.1 Yearly collections of *A. americanum* by life stage and month.

2019													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	35	108	287	99	2	18	0	0	0	0	549
NYMPHS	0	0	0	8	80	24	9	29	14	0	0	0	164
LARVAE	0	0	0	0	40	11	3	1321	284	0	0	0	1659
Ticks collected per month	0	0	35	116	407	134	14	1368	298	0	0	0	2372
2020													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	2	24	118	64	2	10	0	0	0	0	220
NYMPHS	0	0	3	52	399	206	18	29	5	1	0	0	713
LARVAE	0	0	0	0	0	2	91	1838	426	64	0	0	2421
Ticks collected per month	0	0	5	76	517	272	111	1877	431	65	0	0	3354
2021													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	4	15	62	160	31	2	0	0	0	0	274
NYMPHS	0	0	0	1	92	131	102	0	12	0	0	0	338
LARVAE	0	0	0	0	0	2	32	0	0	0	12	0	46
Ticks collected per month	0	0	4	16	154	293	165	2	12	0	12	0	658
2022													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	49	51	231	199	46	39	0	0	0	0	615
NYMPHS	0	0	3	22	188	197	97	68	22	0	0	0	597
LARVAE	0	0	0	0	0	2	427	22	0	0	0	0	451

Table 3.1 (continued)

Ticks collected per month	0	0	52	73	419	398	570	129	22	0	0	0	1663
2019-2022													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	90	198	698	522	81	69	0	0	0	0	1658
NYMPHS	0	0	6	83	759	558	226	126	53	1	0	0	1812
LARVAE	0	0	0	0	40	17	553	3181	710	64	12	0	4577
Ticks collected per month	0	0	96	281	1497	1097	860	3376	763	65	12	0	8047

Table 3.2 Yearly field collections of *A. americanum* by life stage and month.

2019													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	8	88	0	0	1	0	0	0	0	97
NYMPHS	0	0	0	0	34	0	7	15	12	0	0	0	68
LARVAE	0	0	0	0	0	2	0	1304	283	0	0	0	1589
Ticks collected per month	0	0	0	8	122	2	7	1320	295	0	0	0	1754
2020													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	18	28	51	0	0	0	0	0	0	97
NYMPHS	0	0	0	52	36	191	15	14	4	1	0	0	313
LARVAE	0	0	0	0	0	0	91	1789	414	64	0	0	2358
Ticks collected per month	0	0	0	70	64	242	106	1803	418	65	0	0	2768
2021													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	2	0	44	139	23	0	0	0	0	0	208
NYMPHS	0	0	0	0	88	122	98	0	0	0	0	0	308
LARVAE	0	0	0	0	0	2	32	0	0	0	12	0	46
Ticks collected per month	0	0	2	0	132	263	153	0	0	0	12	0	562
2022													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	47	20	29	21	8	0	0	0	0	0	125
NYMPHS	0	0	2	13	119	88	87	0	0	0	0	0	309
LARVAE	0	0	0	0	0	2	421	0	0	0	0	0	423

Table 3.2 (continued)

Ticks collected per month	0	0	49	33	148	111	516	0	0	0	0	0	857
2019-2022													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	49	46	189	211	31	1	0	0	0	0	527
NYMPHS	0	0	2	65	277	401	207	29	16	1	0	0	998
LARVAE	0	0	0	0	0	6	544	3093	697	64	12	0	4416
Ticks collected per month	0	0	51	111	466	618	782	3123	713	65	12	0	5941

Table 3.3 Yearly collections of submitted *A. americanum* by life stage and month.

2019													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	35	100	199	99	2	17	0	0	0	0	452
NYMPHS	0	0	0	8	46	24	2	14	2	0	0	0	96
LARVAE	0	0	0	0	40	9	3	17	1	0	0	0	70
Ticks collected per month	0	0	35	108	285	132	7	48	3	0	0	0	618
2020													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	2	6	90	13	2	10	0	0	0	0	123
NYMPHS	0	0	3	0	363	15	3	15	1	0	0	0	400
LARVAE	0	0	0	0	0	2	0	49	12	0	0	0	63
Ticks collected per month	0	0	5	6	453	30	5	74	13	0	0	0	586
2021													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	2	15	18	21	8	2	0	0	0	0	66
NYMPHS	0	0	0	1	4	9	4	0	12	0	0	0	30
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	0	0	2	16	22	30	12	2	12	0	0	0	96
2022													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	2	31	202	178	38	39	0	0	0	0	490
NYMPHS	0	0	1	9	69	109	10	68	22	0	0	0	288
LARVAE	0	0	0	0	0	0	6	22	0	0	0	0	28

Table 3.3 (continued)

Ticks collected per month	0	0	3	40	271	287	54	129	22	0	0	0	806
2019-2022													
Life Stage of *A. americanum*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	41	152	509	311	50	68	0	0	0	0	1131
NYMPHS	0	0	4	18	482	157	19	97	37	0	0	0	814
LARVAE	0	0	0	0	40	11	9	88	13	0	0	0	161
Ticks collected per month	0	0	45	170	1031	479	78	253	50	0	0	0	2106

Table 3.4 Table listing counties where *A. americanum* was collected from the field vs. from submission.

	Collected from the field		Collected via submission	
Kentucky County List	*A. americanum* collected	*E. chaffeensis* detected	*A. americanum* collected	*E. chaffeensis* detected
Adair County	X	X	X	X
Allen County	X	X	X	
Anderson County	X		X	
Ballard County	X			
Barren County			X	
Bath County	X	X	X	
Bell County			X	
Boone County			X	
Bourbon County	X		X	
Boyd County	X		X	X
Boyle County			X	
Bracken County				
Breathitt County	X			
Breckinridge County	X	X	X	
Bullitt County	X	X	X	X
Butler County			X	
Caldwell County	X	X	X	
Calloway County	X			
Campbell County	X		X	
Carlisle County				
Carroll County	X		X	
Carter County	X	X	X	
Casey County	X	X	X	

Table 3.4 (continued)

Christian County			X	
Clark County	X		X	
Clay County	X	X	X	
Clinton County	X		X	
Crittenden County	X		X	
Cumberland County	X		X	
Daviess County	X		X	
Edmonson County			X	
Elliott County	X	X		
Estill County			X	X
Fayette County			X	X
Fleming County	X		X	
Floyd County			X	
Franklin County	X		X	X
Fulton County				
Gallatin County	X	X	X	
Garrard County	X	X	X	
Grant County			X	X
Graves County	X			
Grayson County	X		X	X
Green County	X	X	X	
Greenup County	X		X	
Hancock County	X			
Hardin County	X	X	X	
Harlan County			X	
Harrison County			X	
Hart County	X	X	X	
Henderson County			X	

Table 3.4 (continued)

Henry County	X		X	
Hickman County	X			
Hopkins County	X		X	
Jackson County	X		X	
Jefferson County	X	X	X	X
Jessamine County	X			
Johnson County	X		X	
Kenton County			X	
Knott County	X	X	X	X
Knox County	X		X	
Larue County	X		X	
Laurel County			X	X
Lawrence County	X	X	X	
Lee County	X		X	X
Leslie County	X	X		
Letcher County			X	
Lewis County	X		X	
Lincoln County			X	
Livingston County	X		X	
Logan County	X		X	
Lyon County	X		X	
Madison County	X	X	X	X
Magoffin County	X			
Marion County	X	X	X	
Marshall County	X		X	
Martin County			X	
Mason County				
McCracken County	X		X	

Table 3.4 (continued)

McCreary County	X		X	
McLean County	X		X	
Meade County	X		X	
Menifee County	X		X	
Mercer County	X	X	X	X
Metcalfe County	X	X	X	X
Monroe County	X	X		
Montgomery County	X		X	X
Morgan County	X	X	X	X
Muhlenberg County	X		X	
Nelson County	X		X	
Nicholas County				
Ohio County	X			
Oldham County	X		X	
Owen County	X	X	X	
Owsley County	X	X		
Pendleton County	X	X	X	X
Perry County	X		X	
Pike County			X	
Powell County	X		X	
Pulaski County	X		X	
Robertson County			X	
Rockcastle County	X		X	
Rowan County	X		X	
Russell County	X		X	
Scott County	X	X	X	X
Shelby County	X		X	
Simpson County			X	

Table 3.4 (continued)

Spencer County	X	X	X	
Taylor County	X	X	X	
Todd County	X			
Trigg County	X		X	
Trimble County	X		X	X
Union County	X			
Warren County	X		X	
Washington County	X	X	X	
Wayne County	X	X		
Webster County	X			
Whitley County	X			
Wolfe County	X			
Woodford County	X		X	X

Figure 3.1 Example of dragging in tick habitat with a close-up of a male *A. americanum*.

This method consists of using a one-meter by one-meter light-colored cloth attached to a dowel rod that is pulled along the ground at a slow pace by an attached rope. Questing ticks attach to the cloth as it passes by them. Ticks are collected from the cloth every ten meters using forceps and placed into collection vials.

Figure 3.2 ***Amblyomma americanum*** **collections per month and life stage for all years of the study.**

Bar chart shows the number of *A. americanum* collected in total per month for all years of the study.

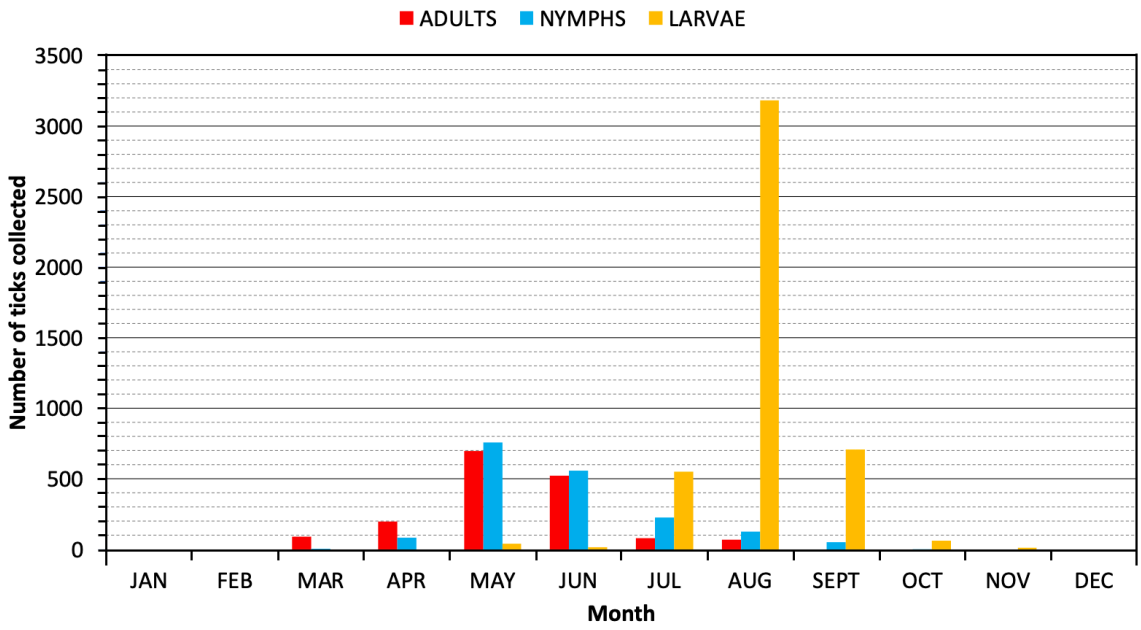


Figure 3.3 *Amblyomma americanum* field collections per month and life stage for all years of the study.

Bar chart shows the number of field-collected *A. americanum* collected in total per month for all years of the study.

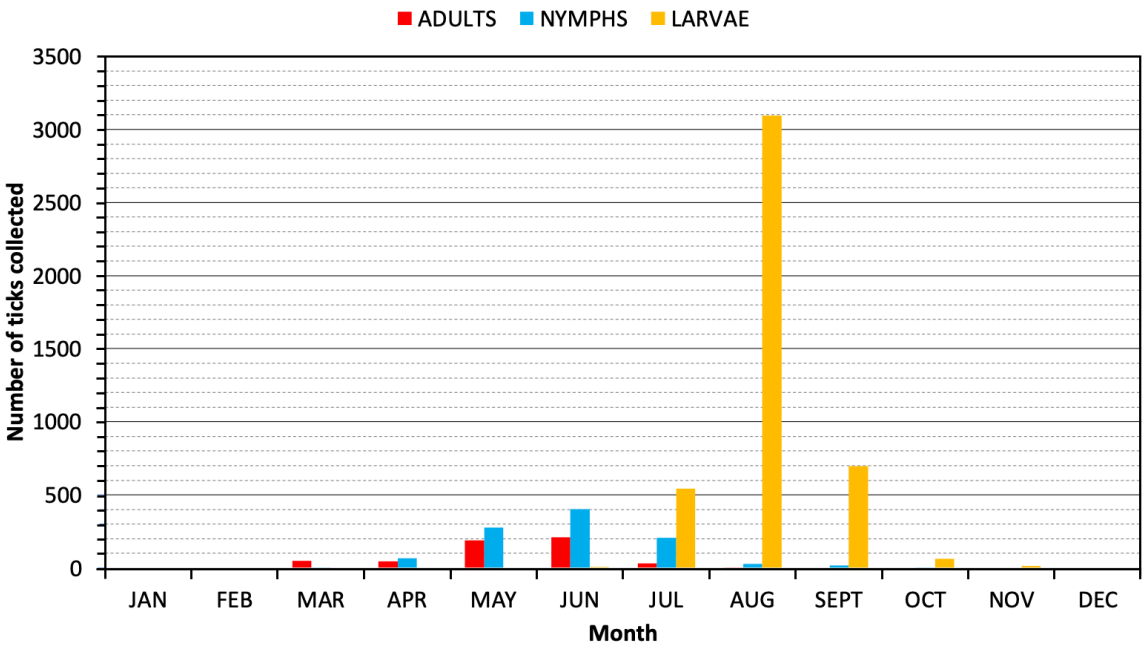


Figure 3.4 ***Amblyomma americanum*** **submitted collections per month and life stage for all years of the study.**

Bar chart shows the number of submitted *A. americanum* collected in total per month for all years of the study.

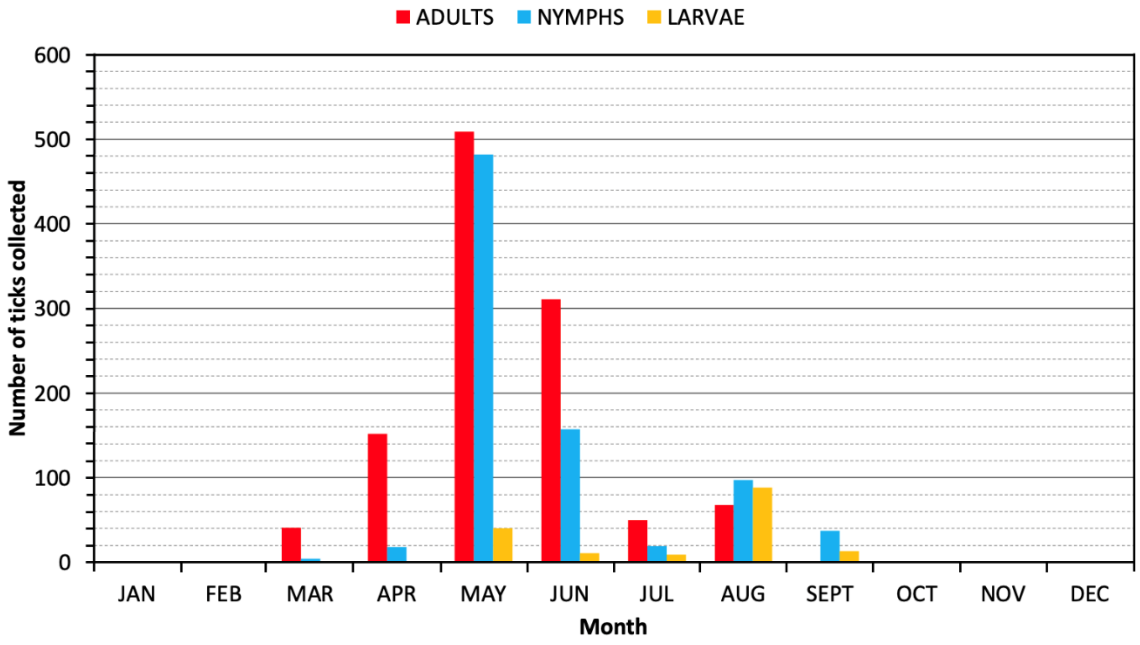


Figure 3.5 County-level distribution of *A. americanum* and *E. chaffeensis* in Kentucky.

Counties shaded grey represent counties where *A. americanum* ticks were collected, either in the field or through submission. Counties with a black dot represent the detection of *E. chaffeensis* in only field-collected ticks from that county, a white dot represents detection in only submitted ticks from that county, and a half-black half-white circle represents detection in both field-collected and submitted ticks from that county.

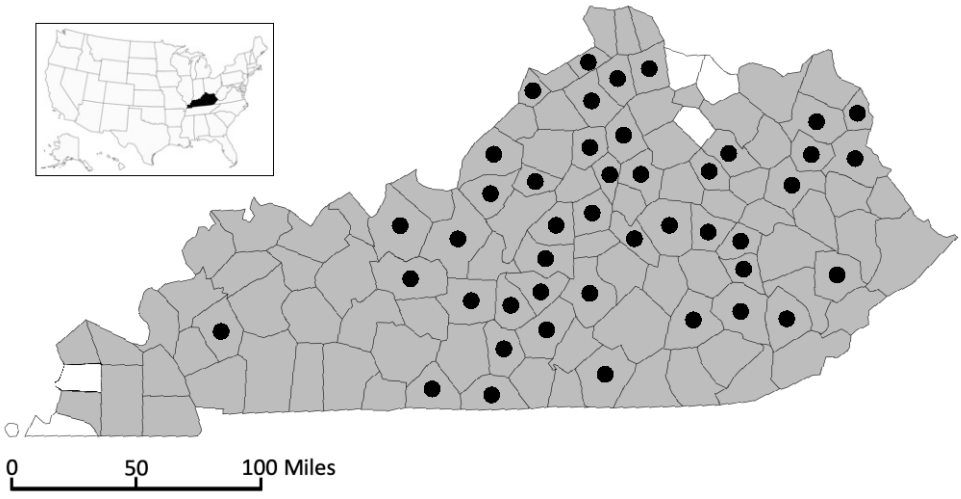
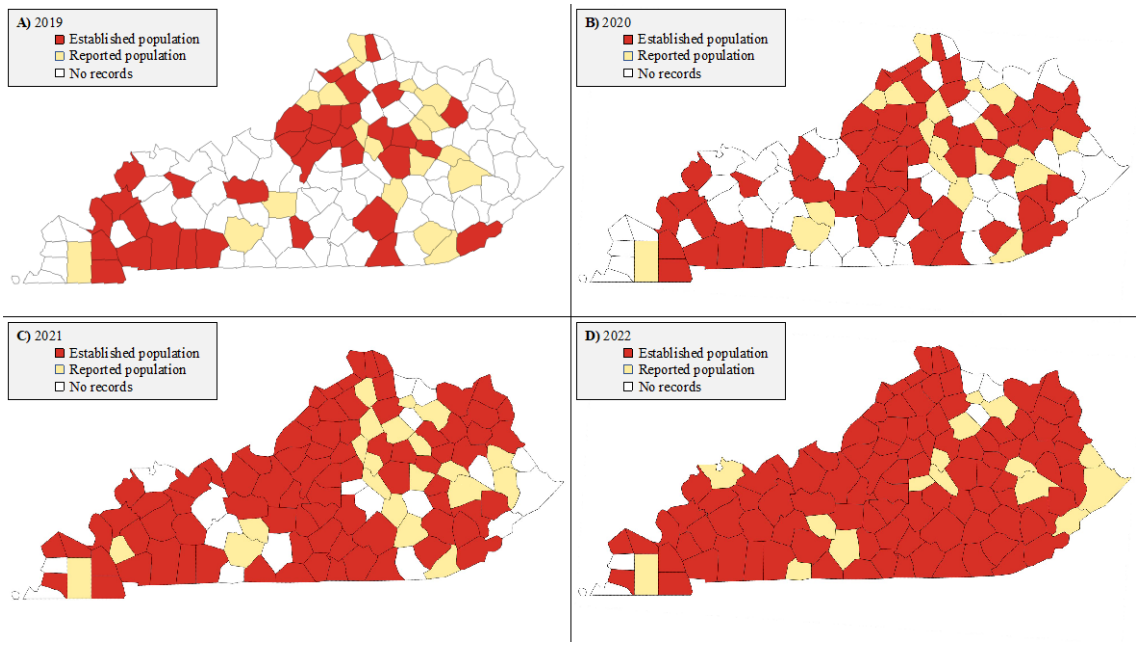


Figure 3.6 County establishment status for each year of this study.

Per guidelines, once a county is classified as “established” it will not revert to a ”reported” or “no records” status, and once a county is classified as “reported” it will not revert to a “no records” status.



CHAPTER 4. DISTRIBUTION OF *DERMACENTOR VARIABILIS* AND *AMBLYOMMA MACULATUM* WITH TESTING FOR TWO SPOTTED FEVER GROUP RICKETTSIA IN KENTUCKY FROM 2019-2022

The following chapter has been submitted for publication in Journal of Medical Entomology.

4.1 Abstract

Spotted fever rickettsioses encompass a group of illnesses with similar clinical symptoms caused by *Rickettsia spp.* bacteria. *Rickettsia rickettsii* is responsible for causing Rocky Mountain spotted fever in humans and is transmitted via the bite of an infected tick. Curiously, while cases of this disease are reported each year, there is a historically low prevalence of this bacterium in its tick vector, *Dermacentor variabilis*. For this reason, it is theorized that other *R. spp.*, namely *R. parkeri,* which is vectored by *Amblyomma maculatum* ticks, may be responsible for causing illness that is being incorrectly diagnosed as Rocky Mountain spotted fever. Here, I report the county-level distribution of *D. variabilis* infected with *R. rickettsii* and *A. maculatum* infected with *R. parkeri* in Kentucky between January 2019 to December 2022. I collected ticks in the environment by dragging and receiving them through the Kentucky Tick Submission Program. I collected 1,176 *D. variabilis* and 26 *A. maculatum* ticks from 99 counties. I report established populations of *D. variabilis* in 44 counties and *A. maculatum* in only one county. I detected *R. rickettsii* (<1%) in three engorged, host-collected *D. variabilis* samples but failed to detect this bacterium in any host-seeking ticks. I did not detect *R. parkeri* in any *A. maculatum* samples tested. Additional surveillance targeted in counties where Rocky Mountain spotted

fever cases are reported would provide more insight into the true prevalence of pathogenic *R. spp* in the tick population.

KEYWORDS: Spotted fever rickettsiosis, *D. variabilis*, *A. maculatum*, tick surveillance, tick-borne pathogen surveillance

4.2 Introduction

Spotted fever rickettsioses are a group of illnesses caused by *Rickettsia spp.* infection. *Rickettsia* is a genus of obligate intracellular bacteria that rely on the ability to invade, grow, and replicate inside living eukaryotic host cells for survival. Due to their nonmotile nature, the bacteria must depend on vectors such as fleas, lice, mites, and ticks for transport from one host to another (Eustis & Fuller, 1952; Reeves et al., 2005; Yazid et al., 2011; Portillo et al., 2017). Even though most *Rickettsia. spp.* are non-pathogenic to vertebrates, some are known for causing noteworthy illnesses such as boutonneuse fever, Rickettsialpox, Rocky Mountain spotted fever, and *R. parkeri* rickettsiosis.

Rocky Mountain spotted fever is perhaps the most well-known spotted fever disease. It is caused by *R. rickettsii*, which is maintained in a zoonotic cycle between its tick vector and host. A notable feature of this bacterium is its ability to preserve via transovarial, in addition to transstadial, transmission, allowing larvae, as well as nymphs and adults, to vector it. Transmission occurs through the bite of an infected tick, with symptoms generally occurring within 14 days of the bite (Dalton et al., 1995). When the bacteria enter a dead-end host, like humans, they invade and damage the cells lining the blood vessels and cause blood leakage. This leakage into the surrounding tissue results in a spotted rash (Hackstadt, 1996). Only three ticks are known to transmit *R. rickettsii*. The primary vector is *Dermacentor variabilis*, the American dog tick, while secondary vectors include *D. andersoni,* the Rocky Mountain wood tick, and *Rhipicephalus sanguineus*, the brown dog tick (Demma et al., 2005; Dantas-Torres, 2007).

Dermacentor variabilis has been collected in most of the eastern and central United States and southern Canada, generally occupying open fields and woodlands (Bishopp & Trembley, 1945; Burg, 2001; Minigan et al., 2018). Although it is the principal vector in

all of North America, *R. rickettsii* prevalence in this species is historically low, even in regions where the disease is endemic (Sonenshine & Mather, 1994). This lack of detection in *D. variabilis* has led to questions regarding how such low levels of infection in the vector could result in so many reports of human cases. Many theorize that previous reports of *R. rickettsii* infections in humans have been misdiagnosed and were, in fact, caused by a different *Rickettsia spp.* infection such as *R. parkeri*.

Rickettsia parkeri was isolated in the 1930s and later designated as a spotted fever group rickettsia (Parker, 1940; Lackman et al., 1949; Lackman et al., 1965). Speculation over whether it could be a cause of illness persisted until Paddock et al. 2004 confirmed the role of this pathogen as a cause of spotted fever rickettsiosis in humans. Transmission occurs via the bite of infected *Amblyomma maculatum*, commonly known as the Gulf Coast tick. This species readily inhabits coastal areas of the southern United States but has been collected further inland as hosts carry it to new areas (Sumner et al., 2007; Trout et al., 2010; Paddock & Goddard, 2015). While not as severe of an infection as *R. rickettsii*, *R. parkeri* rickettsiosis shares many symptoms similar to Rocky Mountain spotted fever and since serological tests cannot differentiate between these two infections, determining which *Rickettsia* is responsible is challenging (Paddock et al., 2008).

The lack of detection of *R. rickettsii* bacteria in *D. variabilis*, coupled with the clinical similarity between these two bacterial infections, prompts the question of whether other *Rickettsia*l organisms such as *R. parkeri*, are responsible for some of the Rocky Mountain spotted fever cases reported in Kentucky previously. Kentucky had high incidence rates for human spotted fever rickettsiosis for several years (Centers for Disease Control and Prevention e, 2022) and considering that Rocky Mountain spotted fever is a

rapidly progressive and potentially fatal tick-borne disease, knowing the prevalence of infection in the tick vector is important for estimating the risk of infection from a tick bite in the area.

Previous reports of tick surveillance in Kentucky document both *D. variabilis* and *A. maculatum* presence but report little detection of *R. rickettsii* or *R. parkeri* in the populations. Here, the objectives were to determine the distributions of 1) *D. variabilis* infected with *R. rickettsii* and 2) *A. maculatum* with *R. parkeri*. Since Kentucky has 120 counties, it was not practical for us to perform active surveillance in every county. Therefore, I utilized passive surveillance through the form of a tick submission program so that I could attempt the collection of data from as many Kentucky counties as possible. Since the study objective was determining the presence/absence of both tick and pathogen, the use of these two methods together was sufficient as outlined in the CDC's "Guide to the Surveillance of Metastriate Ticks (Acari: Ixodidae) and their Pathogens in the United States" (National Center for Emerging and Zoonotic Infectious Diseases, 2020).

4.3 Materials and Methods

4.3.1 Active surveillance

Ticks were collected as described in previous chapters. Briefly, collections occurred via dragging in nature and through the Kentucky Tick Submission Program. Ticks were collected from the field by dragging in publicly accessible areas or on private property (Figures 4.1 & 4.2). The date and time, GPS coordinates, temperature, and habitat type (forest, brush, grassland, forest and brush, forest and grassland, or brush and grassland) were recorded at every sampling event. Ticks were stored in 70% ethanol and stored at -20ºC in the laboratory at the University of Kentucky.

4.3.2 Passive surveillance

Veterinary practices in all 120 of Kentucky's counties were invited to submit ticks removed from animals brought into their clinic between March 2019 through December 2022 as part of the Kentucky Veterinarian Tick Submission Program. Participants were provided instructions on how to package and ship the tick for submission and filled out a form to collect basic contact information as well as the date of collection, county of collection, host species, age, and breed, travel outside the home county in the two weeks preceding, the use of tick preventative medicine (if applicable), habitat type in which the tick was collected and the site ownership type. All specimens were shipped to the University of Kentucky Department of Entomology.

In addition, I accepted ticks from non-veterinary practices, including health departments, physician offices, and the public. With each submission, information forms were included to obtain basic contact information, the date the tick was collected and shipped, county of collection, travel outside the home county in the two weeks preceding, habitat type in which the tick was believed to be collected, site ownership type, host type, and whether the tick was attached or not. Directions on packaging and shipping the tick were also included. Samples from outside the state of Kentucky or instances when the date and/or county-level location information could not be provided were not accepted. All ticks received through these submission programs were stored under the same conditions as ticks collected from the environment.

4.3.3 Tick identification

Ticks were identified and sexed morphologically by comparison with standard keys in the laboratory at the University of Kentucky (Keirans et al. 1989; Egizi et al. 2019).

Ticks identified as anything other than the target organisms, *D. variabilis* and *A. maculatum*, were omitted from this chapter and included in other chapters.

4.3.4 DNA Extraction

For DNA extraction, individual ticks were bead-beaten with 2.0 mm Zirconia beads from BioSpec Products in a Tissueminser (MP Biomedicals) at a speed of 0.6 m/s for three consecutive cycles of 40 s each to ensure proper lysis. The homogenate for each tick in the sample was then pooled. DNA extraction was performed using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturer's instructions. Since the objective was to determine the presence/absence of the pathogens, I pooled ticks for testing. Ticks were not combined from multiple submissions/collection events, hosts, or locations for testing. Individual tick testing was not performed.

4.3.5 Detection of *R. rickettsii* and *R. parkeri*

The presence of *R. rickettsii* in *D. variabilis* ticks was detected using primers RR1370F (ATAACCCAAGACTCAAACTTTGGTA) and RR1494R (GCAGTGTTACCGGGATTGCT). *R. parkeri* in *A. maculatum* ticks was detected using primers Rpa129F (CAAATGTTGCAGTTCCTCTAAA) and Rpa224R (AAAACAAACCGTTAAAACTACCG) (Gaines et al., 2014). Positive controls for *R. rickettsii* and *R. parkeri* came from the Centers for Disease Control and Prevention, *Rickettsia*l Isolate Repository Collection. Negative controls used included non-target DNA and no DNA (nuclease-free water). Ten microliter qPCR reactions containing 5 µL SYBR Green Master Mix (Bio-Rad), 2 µL extracted DNA, 0.5 µL of 0.4µM forward primer, 0.5 µL of 0.4µM reverse primer, and 2 µL nuclease-free water were run at the following PCR conditions: 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, and 61°C for 1 min.

The presence of expected PCR fragment in the positive samples was verified by running PCR products on a 1.5% agarose gel (Figure 4.8).

4.3.6 Determination of county establishment status

In accordance with the Centers for Disease Control and Prevention's "Guide to the Surveillance of Metastriate Ticks (Acari: Ixodidae) and their Pathogens in the United States", I classified county status based on the county-level establishment criteria (National Center for Emerging and Zoonotic Infectious Disease, 2020). As directed, a county may be designated as "established" for a tick species when ≥ six ticks of a single life stage or > one life stage of a single species are collected from the county within 12 months. A county may be designated as "reported" for a tick species when < six ticks of a single life stage are collected from the county within 12 months. Since there is greater confidence in the presence of a species rather than the absence of a species, once a county is classified as "established" it will not revert to a "reported" or "no records" status. Similarly, once a county is classified as "reported" it will not revert to a "no records" status.

4.4 Results

4.4.1 Summary

I collected 1,176 *D. variabilis* (1167 adults, eight nymphs, one larva) and 26 adult *A. maculatum* from 99 counties between January 2019 and December 2022 (Figure 4.6; Tables 4.1 & 4.4). *Rickettsia rickettsii* was detected in three samples of engorged *D. variabilis* from separate counties, but no *R. parkeri* was detected in the *A. maculatum* ticks in this study (Figure 4.7). *Dermacentor variabilis* was collected from March to September, while *A. maculatum* was collected in May and August (Figure 4.3). Seasonality differed slightly between years and collection methods for *D. variabilis*, but *A. maculatum* was only

collected once in 2021 and three times in 2022, so there is not enough data to confidently report seasonality. I report 44 established and 55 reported counties for *D. variabilis* and one established and three reported counties for *A. maculatum* (Figures 4.9 & 4.10).

4.4.2 Active surveillance

I collected 312 adult *D. variabilis* ticks in the field from 59 of the 106 counties dragged for ticks during the study period (Table 4.2). Ticks were collected primarily from grassland (24.66%) and mixed grassland and forest habitats (24.22%) but were also collected in mixed grassland and brush (18.39%), mixed forest and brush (16.59%), forest (13.00%), and brush (3.14%) habitats. Collection occurred between 50° and 89° Fahrenheit as early as March and as late as August (Figure 4.4). Peak activity from field collections was seen in May and June.

I collected five adult *A. maculatum* ticks in the field from one of the 106 counties dragged for ticks during the study period (Table 4.5). The site where collection occurred was the John C Williams Wildlife Management Area in Nelson County, a grassland/wetland habitat, in May of 2021. The recorded temperature was 75° Fahrenheit.

4.4.3 Passive surveillance

Through the submission program, I collected 864 *D. variabilis* ticks (855 adults, eight nymphs, and one larva) (Table 4.3). Out of the 1,107 submissions received through Kentucky Tick Submission Program during the study period, 435 submissions (39.3%) contained *D. variabilis* ticks. Ticks were submitted from cat, cow, dog, horse, and human hosts for *D. variabilis*. A few submissions containing *D. variabilis* failed to include the host type and species; therefore, the host type was classified as an “undetermined animal”. I accepted submissions from all 120 of Kentucky’s counties and received submissions

containing *D. variabilis* from 81 counties (Table 4.5). This species was received from April to August each year except for a single tick submitted in September of 2021. Ticks were received in the highest abundance in May (Figure 4.5).

Twenty-one *A. maculatum* adults were submitted from three counties (<1% of all submissions) (Table 4.5). Ticks were submitted from dog and human hosts. Ticks were received in May and August of 2022.

4.4.4 Detection of *R. rickettsii* and *R. parkeri*

Rickettsia rickettsii was detected in *D. variabilis* samples from three counties (Floyd, Grayson, and Rockcastle counties) in Kentucky (Figure 4.7). Only 3 of 485 (0.6%) *D. variabilis* samples tested were positive for *R. rickettsii.* These three samples contained host-collected, blood-fed American dog ticks; thus, minimal infection rate (as calculated in previous chapters) can not apply here. No *R. rickettsii* was detected in unfed ticks collected from nature. I did not detect any *R. parkeri* in *A. maculatum* tested in this study.

4.4.5 County establishment status

I made progress establishing Kentucky counties' statuses every year of the study. Of the 99 counties where I collected *D. variabilis*, I were able to classify 44 as established counties and 55 as reported counties for this species (Figure 4.9). In 2019, I classified 14 counties as established and 24 counties as reported. I classified an additional eight counties as established and an additional 12 counties as reported in 2020, an additional four counties as established and an additional 15 counties as reported in 2021, and then an additional 18 counties as established and an additional 20 counties as reported in 2022. I classified 21 counties with "no records" due to the lack of collection. For *A. maculatum*, I was able to classify one established county and three reported counties (Figure 4.10).

4.5 Discussion

The primary objectives of this study were to document the county-level distribution of *D. variabilis* infected with *R. rickettsii* and *A. maculatum* infected with *R. parkeri* in Kentucky. I implemented both active and passive surveillance systems to ensure the collection of as many ticks as possible.

The presence of *D. variabilis* in Kentucky is dated as far back as the 1940s (Bishopp & Trembley, 1945; Mount & Snoddy, 1983; Burg, 2001; Fritzen et al., 2011; Pagac et al., 2014; Lockwood et al., 2018; Slabach et al., 2018; Hecht et al., 2019). I collected *D. variabilis* from 99 counties over four years of surveillance, with established populations in 44 counties. I identified this species in over a third (39.2%) of all samples submitted through the Kentucky Tick Submission Program from various hosts, including common domesticated animals like cats and dogs, livestock, and humans. My surveillance data shows that *D. variabilis* is a widespread tick species commonly encountered across Kentucky. This is further supported by its frequent appearance in other tick collections performed in the state previously; it was the most collected species by Fritzen et al. 2011 and the second-most collected species after *D. albipictus* in a study by Slabach et al. 2018. It is also found in high abundance in neighboring states Tennessee and Virginia (Moncayo et al., 2010; Cumbie et al., 2021). The distribution reported here adds to what is currently published in the CDC American Dog Tick Surveillance database. I report an additional 14 established and an additional 36 reported counties compared to this national database (Centers for Disease Control and Prevention b, 2022).

Amblyomma maculatum is frequently collected in the southeastern United States. Previous collections of this tick are documented in eastern Kentucky by Slabach et al. 2018 and then in ten counties across Kentucky by Lockwood et al. 2018. A national database

mapping *A. maculatum* presence is not publicly available, as is for *I. scapularis*, *I. pacificus*, *A. americanum*, and *D. variabilis*. However, the four counties I collected from (Daviess, Jefferson, Laurel, and Nelson) were found to not be previously reported in prior publications, thus suggesting this may be the first documentation of Gulf Coast ticks in these counties. The sample size of *A. maculatum* ticks in this study is small, limiting distribution, habitat, and seasonality analysis. Since surveillance was not targeted toward any species, I theorize that while the Gulf coast tick is certainly present in Kentucky, it may exist in lower numbers than the other species investigated in this dissertation. Previous surveys outside Kentucky report *A. maculatum* in warm, xeric habitats such as prairies and coastal plains (Cooley & Kohls, 1944; Nadolny & Gaff 2018). Interestingly, this study's single field collection *of A. maculatum* occurred in a wetland nature preserve in Nelson County. Additional surveillance targeting the Gulf Coast tick is needed to make better conclusions about its habits in Kentucky.

The five-year *D. variabilis* surveillance performed by J. G. Burg from 1992 to 1996 in the bluegrass region of Kentucky provides, perhaps, the best look at the local seasonality of this species. He found that adults emerged in April with bimodal host-seeking activity each year; first from mid-April to May and then again in late June and early July. Activity ceased in August (Burg, 2001). Here, I collected *D. variabilis* as early as March, at the highest abundance in May and June, and as late as September. Field collections in March and April were low compared to May and June, while submissions increased from April to May and then declined through September. While the seasonality reported here does not vary significantly from that reported in Burg, 2001, there is a slightly longer activity season seen in this study while the monthly surveillance from 2015-2017 in Oklahoma found

comparable seasonality (Noden et al., 2022). All *A. maculatum* and *D. variabilis* collections occurred in warm months. Late spring and summer months appear to present the greatest risk of exposure to ticks and, therefore, tick bites, therefore, increased awareness and prevention campaigns should be conducted during this time of the year.

Human cases of spotted fever rickettsiosis are more common in the southern and western regions of Kentucky, with a 2019 incidence rate of 77.67 per million people (Centers for Disease Control and Prevention e, 2022). Specifically, in the counties of Floyd, Grayson, and Rockcastle the incidence rates of cases per million people are 7.02, 33.11, and 14.97, respectively, for the years 2011-2019 (Kentucky Department for Public Health 2021). I failed to detect this pathogen in any questing ticks. The lack of *R. rickettsii* in questing *D. variabilis* is consistent with many other studies that either failed to detect this pathogen or found it at very low (<1%) levels. I did detect *R. rickettsii* in three samples of engorged host-collected ticks. In all cases, travel outside of the county in the 14 days prior to detection of the tick was ruled out. Therefore, it is unlikely that the ticks were picked up elsewhere but does not indicate that the infected ticks obtained the infection in the county. Whether or not the disease occurred in the hosts is unknown. To effectively control the transmission of *R. rickettsii*, further surveillance in these three counties should focus on collecting *D. variabilis* as well as tissue specimens from potential hosts. Since *R. rickettsii* can be passed on transovarially it may be well sustained through a healthy tick population, in which case control efforts focused on reducing the survivability of the tick (i.e., habitat modification) would be more effective than mitigating host accessibility (i.e., application of acaricides). However, if the pathogen is present in reservoir hosts, targeting both tick and host would be beneficial to comprehensively control transmission.

I did not detect any *R. parkeri,* which may be due to the small sample size. *Rickettsia parkeri* infection in *A. maculatum* ticks from Kentucky has been reported in previous surveys. Pagac et al. 2014 reported an infection rate of 14.3%, and Lockwood et al. 2018 reported a 3% infection rate. Moreover, Jiang et al. 2012 found *R. parkeri*-infected *A. maculatum* from multiple sites in Kentucky. I hypothesize that the lack of *R. parkeri* detection in this study can be attributed to the small sample size since *R. parkeri* has been documented in Kentucky's Gulf Coast tick populations repeatedly.

Limitations of this study include 1) uneven sample sizes across counties and 2) an insufficient number of *A. maculatum* ticks for *R. parkeri* screening and analysis. While I attempted to collect in as many counties as possible, it was not feasible for us to actively survey for ticks in each of Kentucky's 120 counties and repeat collections over the study period in all sample sites. However, even with these limitations, I was able to achieve the objectives of this study. A significant contribution is the report of tick presence statewide and the detection of *D. variabilis* and *A. maculatum* in counties not previously noted. An article published in 2020 analyzing records from the U.S. National Tick Collection and National Ecological Observatory Network databases reported data for *D. variabilis* populations in only six counties in Kentucky; established populations were documented from Calloway, Hardin, and Muhlenberg counties and reported populations in Christian, Fayette, and Hart (Lehane et al., 2020). Further, as mentioned, the current county distribution reported through the Center for Disease Control and Prevention's American dog tick surveillance program reports only 52 counties with known populations. Here, I report additional counties not included in the database. Moreover, the counties where *A. maculatum* collection occurred are not previously documented in publications.

From January 2019 to December 2022, I performed surveillance for ticks and select tick-borne pathogens in Kentucky. Here, I report my findings for *D. variabilis* infected with *R. rickettsii* and *A. maculatum* infected with *R. parkeri*. I collected *D. variabilis* from several counties across Kentucky, indicating that this is a well-established and widespread tick species in the state. The sample size of *A. maculatum* is small and the low numbers could explain the lack of *R. parkeri* detection. While I did detect *R. rickettsii*, it was only detected in samples containing engorged, host-collected ticks.

Table 4.1 Yearly collections of *D. variabilis* by life stage and month.

2019													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	48	72	28	10	34	0	0	0	0	192
NYMPHS	0	0	0	1	4	1	0	0	0	0	0	0	6
LARVAE	0	0	0	0	0	1	0	0	0	0	0	0	1
Ticks collected per month	0	0	0	49	76	30	10	34	0	0	0	0	199
2020													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	3	106	87	21	7	0	0	0	0	224
NYMPHS	0	0	0	0	0	0	0	0	0	0	0	0	0
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	0	0	0	3	106	87	21	7	0	0	0	0	224
2021													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	72	51	66	37	1	1	0	0	0	228
NYMPHS	0	0	0	0	0	0	0	0	0	0	0	0	0
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	0	0	0	72	51	66	37	1	1	0	0	0	228
2022													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	4	21	207	173	80	38	0	0	0	0	523
NYMPHS	0	0	0	0	1	1	0	0	0	0	0	0	2
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4.1 (continued)

Ticks collected per month	0	0	4	21	208	174	80	38	0	0	0	0	525
2019-2022													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	4	144	436	354	148	80	1	0	0	0	1167
NYMPHS	0	0	0	1	5	2	0	0	0	0	0	0	8
LARVAE	0	0	0	0	0	1	0	0	0	0	0	0	1
Ticks collected per month	0	0	4	145	441	357	148	80	1	0	0	0	1176

Table 4.2 Yearly field collections of *D. variabilis* by life stage and month.

2019													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	0	8	1	3	28	0	0	0	0	40
NYMPHS	0	0	0	0	0	0	0	0	0	0	0	0	0
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	0	0	0	0	8	1	3	28	0	0	0	0	40
2020													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	1	84	52	8	1	0	0	0	0	146
NYMPHS	0	0	0	0	0	0	0	0	0	0	0	0	0
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	0	0	0	1	84	52	8	1	0	0	0	0	146
2021													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	0	29	25	14	0	0	0	0	0	68
NYMPHS	0	0	0	0	0	0	0	0	0	0	0	0	0
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	0	0	0	0	29	25	14	0	0	0	0	0	68
2022													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	4	3	0	44	7	0	0	0	0	0	58
NYMPHS	0	0	0	0	0	0	0	0	0	0	0	0	0
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4.2 (continued)

Ticks collected per month	0	0	4	3	0	44	7	0	0	0	0	0	58
2019-2022													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	4	4	121	122	32	29	0	0	0	0	312
NYMPHS	0	0	0	0	0	0	0	0	0	0	0	0	0
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	0	0	4	4	121	122	32	29	0	0	0	0	312

Table 4.3 Yearly collections of submitted *D. variabilis* by life stage and month.

2019													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	48	64	27	7	6	0	0	0	0	152
NYMPHS	0	0	0	1	4	1	0	0	0	0	0	0	6
LARVAE	0	0	0	0	0	1	0	0	0	0	0	0	1
Ticks collected per month	0	0	0	49	68	29	7	6	0	0	0	0	159
2020													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	2	22	35	13	6	0	0	0	0	78
NYMPHS	0	0	0	0	0	0	0	0	0	0	0	0	0
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	0	0	0	2	22	35	13	6	0	0	0	0	78
2021													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	72	22	41	23	1	1	0	0	0	160
NYMPHS	0	0	0	0	0	0	0	0	0	0	0	0	0
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Ticks collected per month	0	0	0	72	22	41	23	1	1	0	0	0	160
2022													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	18	207	129	73	38	0	0	0	0	465
NYMPHS	0	0	0	0	1	1	0	0	0	0	0	0	2
LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4.3 (continued)

Ticks collected per month	0	0	0	18	208	130	73	38	0	0	0	0	467
2019-2022													
Life Stage of *D. variabilis*	**Month of the year**												**Ticks collected per life stage**
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	
ADULTS	0	0	0	140	315	232	116	51	1	0	0	0	855
NYMPHS	0	0	0	1	5	2	0	0	0	0	0	0	8
LARVAE	0	0	0	0	0	1	0	0	0	0	0	0	1
Ticks collected per month	0	0	0	141	320	235	116	51	1	0	0	0	864

Table 4.4 Table listing counties where *D. variabilis* and *A. maculatum* were collected from the field vs. from submission.

	Collected from the field		**Collected via submission**	
Kentucky County List	*D. variabilis* collected	*A. maculatum* collected	*D. variabilis* collected	*A. maculatum* collected
Adair County	X		X	
Allen County	X		X	
Anderson County			X	
Ballard County				
Barren County	X		X	
Bath County			X	
Bell County			X	
Boone County			X	
Bourbon County	X		X	
Boyd County			X	
Boyle County			X	
Bracken County	X		X	
Breathitt County				
Breckinridge County			X	
Bullitt County	X		X	
Butler County				
Caldwell County	X			
Calloway County			X	
Campbell County	X		X	
Carlisle County				
Carroll County			X	
Carter County	X		X	

Table 4.4 (continued)

Casey County	X		X	
Christian County			X	
Clark County			X	
Clay County			X	
Clinton County			X	
Crittenden County	X			
Cumberland County	X			
Daviess County	X		X	X
Edmonson County				
Elliott County	X			
Estill County			X	
Fayette County	X		X	
Fleming County	X			
Floyd County			X	
Franklin County	X		X	
Fulton County				
Gallatin County	X		X	
Garrard County			X	
Grant County	X		X	
Graves County	X		X	
Grayson County			X	
Green County	X		X	
Greenup County	X		X	
Hancock County	X			
Hardin County			X	
Harlan County				
Harrison County	X		X	
Hart County				

Table 4.4 (continued)

Henderson County	X		X	
Henry County	X		X	
Hickman County				
Hopkins County			X	
Jackson County	X		X	
Jefferson County	X		X	X
Jessamine County			X	
Johnson County				
Kenton County	X		X	
Knott County			X	
Knox County				
Larue County			X	
Laurel County			X	X
Lawrence County			X	
Lee County	X			
Leslie County				
Letcher County	X		X	
Lewis County			X	
Lincoln County			X	
Livingston County			X	
Logan County	X			
Lyon County	X		X	
Madison County			X	
Magoffin County	X			
Marion County	X		X	
Marshall County	X		X	
Martin County			X	
Mason County	X			

Table 4.4 (continued)

McCracken County	X		X	
McCreary County	X		X	
McLean County				
Meade County			X	
Menifee County				
Mercer County	X		X	
Metcalfe County	X			
Monroe County	X			
Montgomery County				
Morgan County	X			
Muhlenberg County				
Nelson County	X	X	X	
Nicholas County	X		X	
Ohio County	X		X	
Oldham County	X		X	
Owen County	X		X	
Owsley County	X			
Pendleton County	X		X	
Perry County			X	
Pike County			X	
Powell County			X	
Pulaski County				
Robertson County	X		X	
Rockcastle County			X	
Rowan County	X		X	
Russell County	X			
Scott County			X	
Shelby County			X	

Table 4.4 (continued)

Simpson County				
Spencer County			X	
Taylor County			X	
Todd County	X			
Trigg County				
Trimble County			X	
Union County				
Warren County	X		X	
Washington County	X		X	
Wayne County				
Webster County	X			
Whitley County	X			
Wolfe County			X	
Woodford County	X		X	

Figure 4.1 ***Dermacentor variabilis*** **adult male (left) and female (right).**

Figure 4.2 ***Ambylomma maculatum*** **male questing on vegetation at the John C Williams Wildlife Management Area in Nelson County.**

Figure 4.3 *Dercamentor variabilis* collections per month and life stage for all years of the study.

Bar chart shows the number of *D. variabilis* collected in total per month for all years of the study.

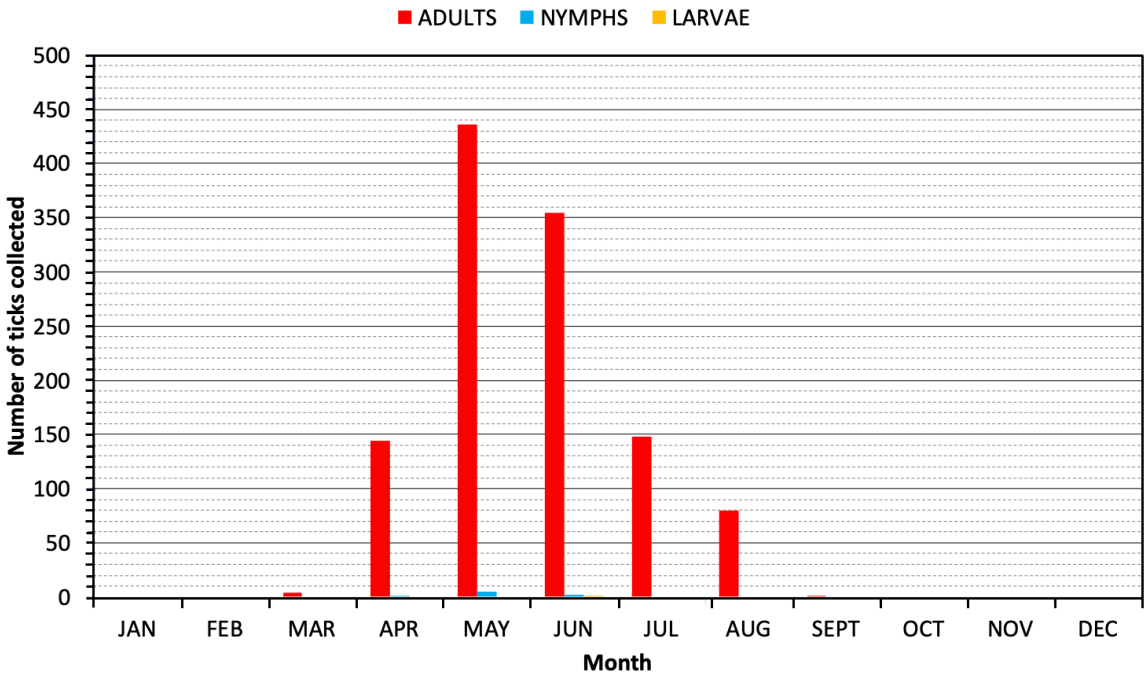


Figure 4.4 *Dermacentor variabilis* field collections per month and life stage for all years of the study.

Bar chart shows the number of field-collected *D. variabilis* collected in total per month for all years of the study.

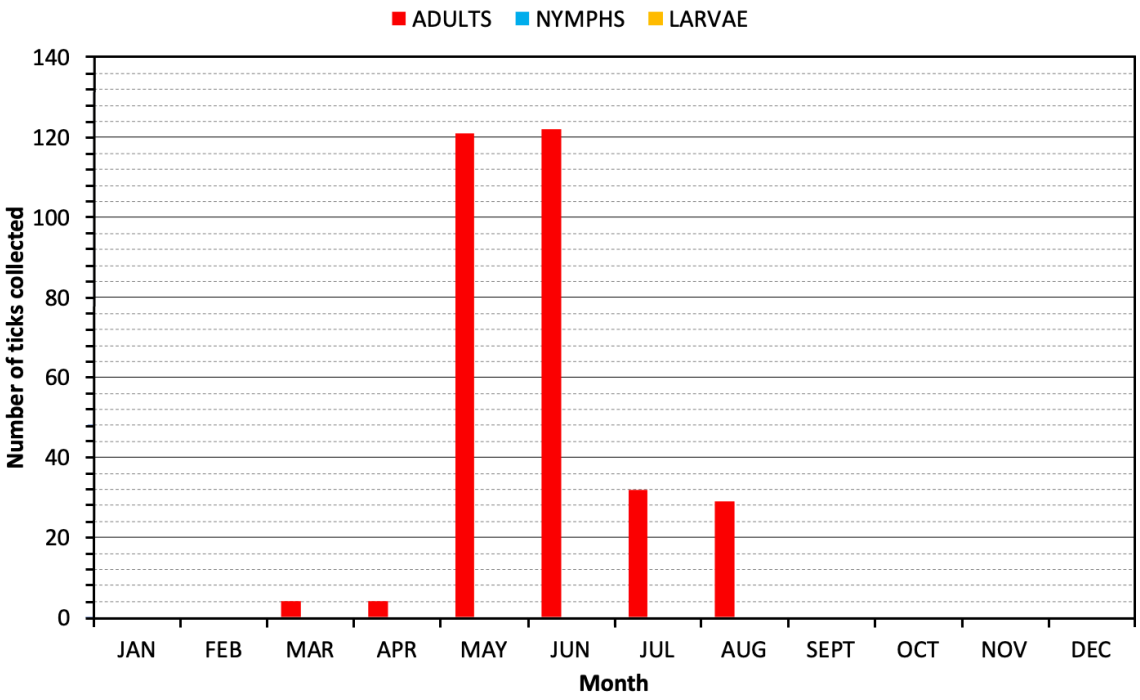


Figure 4.5 *Dermacentor variabilis* submitted collections per month and life stage for all years of the study.

Bar chart shows the number of submitted *D. variabilis* collected in total per month for all years of the study.

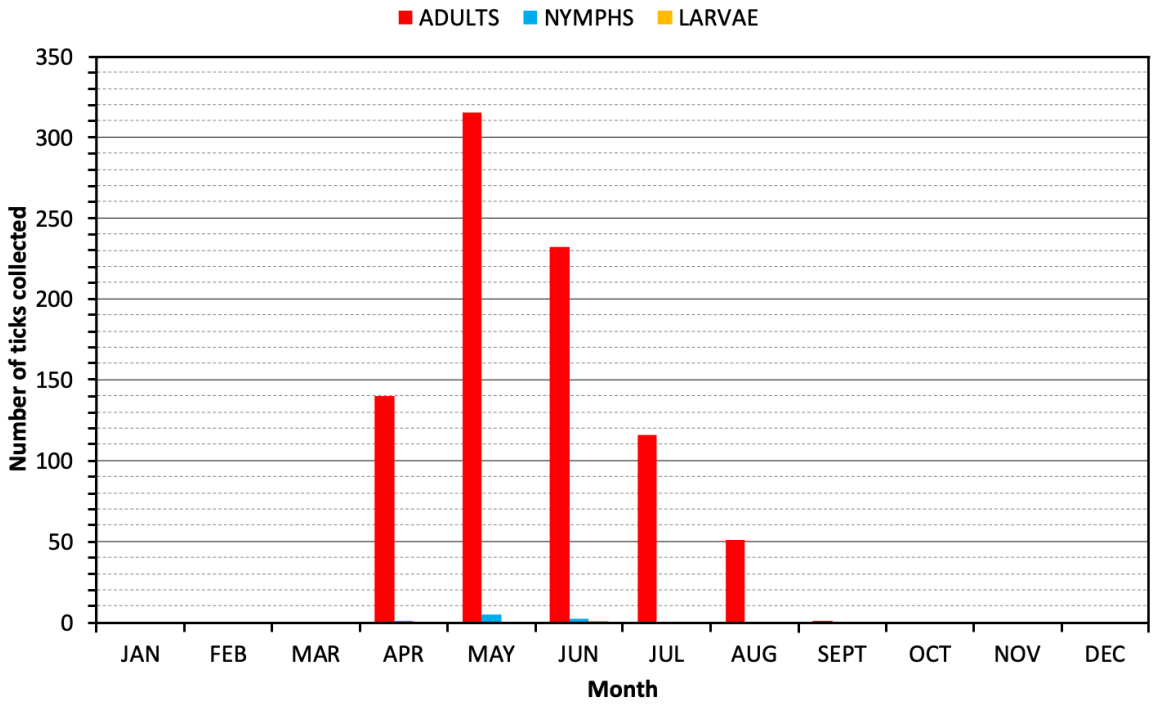


Figure 4.6 Kentucky county distribution of *D. variabilis* and *A. maculatum* collected from this study.

Counties where *D. variabilis* were collected are shown in solid gray. Counties where *A. maculatum* were collected are shown in checkered grey and black. Counties where neither species were collected are shown in solid white.

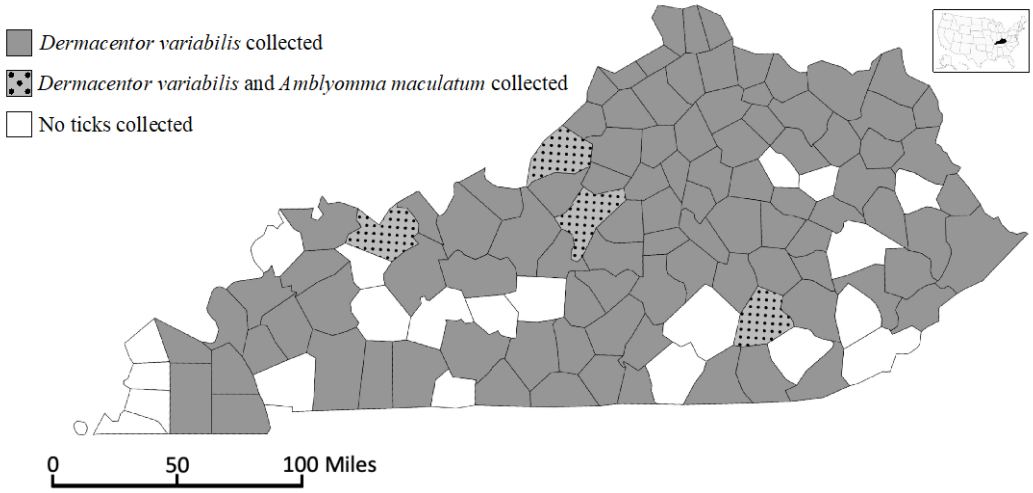


Figure 4.7 Submission information and location in Kentucky for the three samples that *R. rickettsii* was detected in.

For each submission, information was collected using the appropriate submission form. Information for the three samples that were positive for *R. rickettsii* are detailed in this figure.

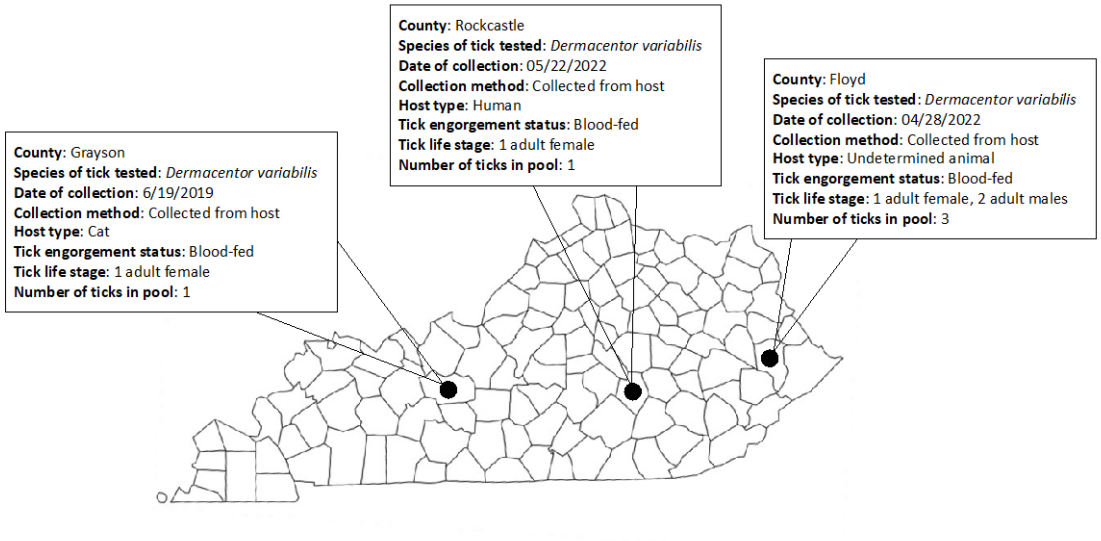


Figure 4.8 Agarose gel electrophoresis (1.5%) verification image of the *R. rickettsii* PCR products.

Lane 1: 1 kb+ DNA ladder. Lane 2: positive control. Lanes 3-5: three DNA samples from Grayson, Floyd, and Rockcastle. Lane 6: negative control.

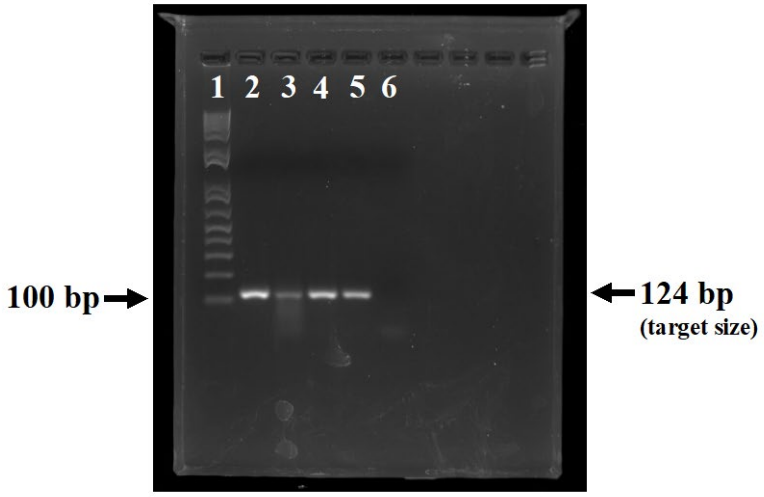


Figure 4.9 County establishment status for *D. variabilis* each year of this study.

Per guidelines, once a county is classified as “established” it will not revert to a ”reported” or “no records” status, and once a county is classified as “reported” it will not revert to a “no records” status.

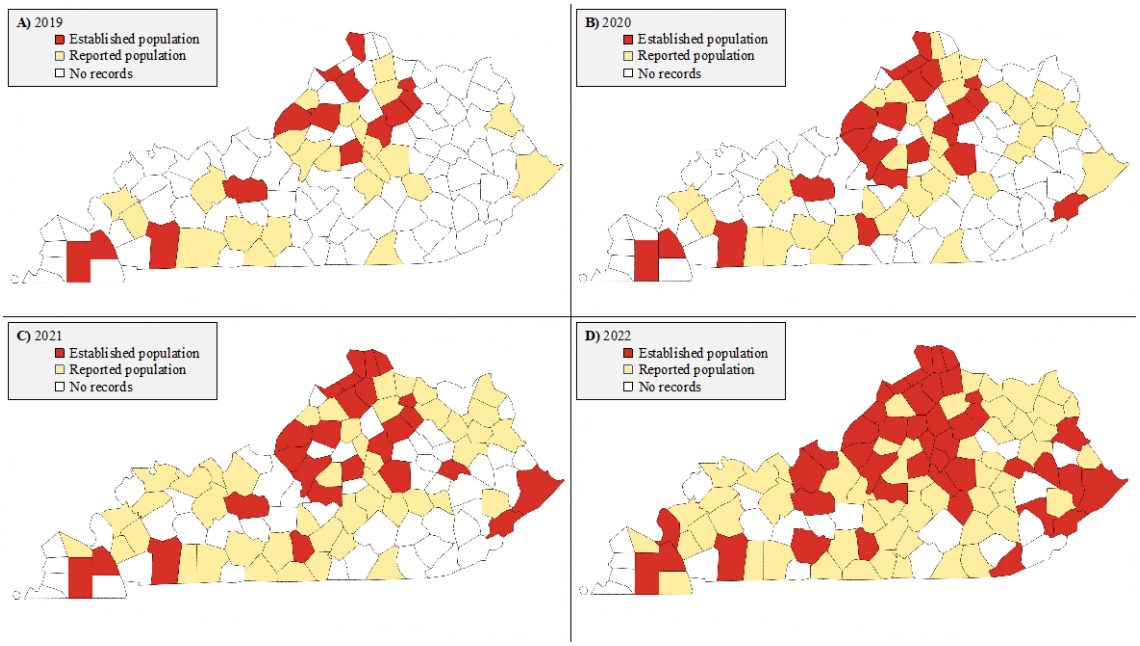
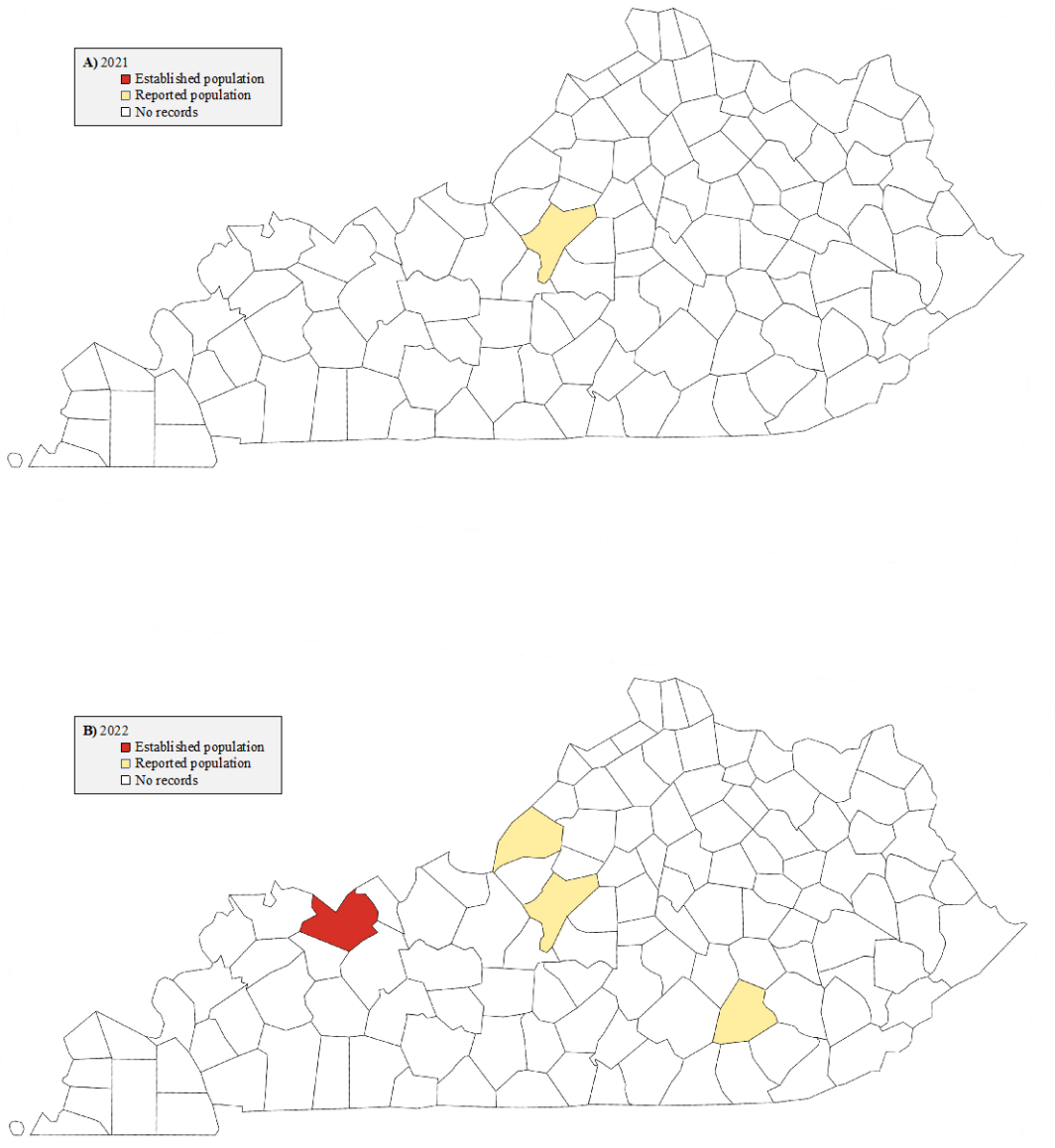


Figure 4.10 County establishment status for *A. maculatum* each year of its collection. Per guidelines, once a county is classified as “established” it will not revert to a ”reported” or “no records” status, and once a county is classified as “reported” it will not revert to a “no records” status.



CONCLUSIONS AND FUTURE STUDIES

The escalating incidence of tick-borne diseases is a growing concern, and despite not being a new threat, the significant rise in human cases over the past two decades highlights the need for increased research into the distribution and prevalence of tick vectors and pathogens they transmit. In Kentucky, the number of human cases of various tick-borne diseases has surged, yet efforts to coordinate a robust surveillance system to monitor ticks and tick-borne pathogens were lacking. In response to this, my dissertation research aimed to determine the distribution, abundance, and seasonality of tick vectors, and the presence and estimated prevalence of select tick-borne pathogens in Kentucky. Emphasis was placed on the dynamics of *Ixodes scapularis* and *Borrelia burgdorferi*, *Ambylomma americanum* and *Ehrlichia chaffeensis*, *Dermacentor variabilis* and *Rickettsia rickettsii*, and *A. maculatum* and *R. parkeri*.

Between January 2019 and December 2022, I collected over 10,000 ticks and identified nine separate species: *A. americanum, A. maculatum, D. albipictus, D. variabilis, Haemaphysalis leporispalustris, H. longicornis, I. brunneus, I. scapularis,* and *Rhipicephalus sanguineus*. The three most abundant species, *A. americanum*, *D. variabilis,* and *I. scapularis*, as well as *A. maculatum,* are vectors for the three most common tick-borne diseases reported in Kentucky. As such, they are the focus of my dissertation chapters.

Amblyomma americanum, the lone star tick, was the most abundant and widespread tick species reported in this study. Eight-thousand forty-seven ticks were collected from 115 counties, with 1,658 adults, 1,812 nymphs, and 4,577 larvae recorded. In the ticks tested, *E. chaffeensis* was detected in samples from 44 counties with a minimal infection

rate (MIR) of 2.2%. Specifically, it was detected in host-seeking ticks from 33 counties, indicating that this pathogen is likely well-sustained in the environment throughout Kentucky. The collection of *A. americanum* occurred from March to November with slight variations in seasonality observed between each year. Adults and nymph collections peaked in May and June while larval collections peaked in August, demonstrating bimodal seasonal activity. Overall, I conclude that *A. americanum* is a widespread, frequently encountered tick species that emergences in March, with peak adult and nymph activity in May and June, while larval activity peaks in August. *Ehrlichia chaffeensis* transmission via this tick's bite should be considered a very possible public health concern, and tactics to prevent exposure should focus efforts during the early spring to early fall.

Dermacentor variabilis was the second most abundant and widespread species identified in this study, though it only accounted for 12% of all ticks surveyed, while *A. maculatum* was scarcely collected. I identified 1,176 *D. variabilis* ticks (1,167 adults, eight nymphs, and one larva) and 26 adult *A. maculatum* ticks from 99 counties. *Rickettsia rickettsii*, the agent for Rocky Mountain spotted fever, was detected in three samples of engorged American dog ticks from separate counties (MIR = <1%), but no *R. parkeri* was detected in any samples. *Dermacentor variabilis* was collected from March to September, while *A. maculatum* was collected in May and August. Seasonality varied slightly between years and collection methods for *D. variabilis,* but *A. maculatum* was only collected once in 2021 and three times in 2022. In general, *D. variabilis* is a prevalent and abundant tick species in Kentucky, with the risk of exposure existing between March and September and heightened risk in April. However, further data is needed to make definitive statements about *A. maculatum.* While spotted fever rickettsiosis is the most frequently reported tick-

borne disease in Kentucky, my data suggests that other *Rickettsia* species, beyond *R. rickettsii* and *R. parkeri*, may be responsible.

Ixodes scapularis was the third most abundant species in this study with 674 ticks collected from 58 counties. The majority of collections are adults. *Borrelia burgdorferi* was detected in ticks from 16 counties with a MIR of 4.6% and detected specifically in host-seeking ticks from seven counties. This detection in ticks collected from the environment indicates that, while seemingly less so than *E. chaffeensis*, this pathogen is well-sustained in the environment of Kentucky. *Ixodes scapularis* was collected every month of the year with variations between years and collection methods and a notable increase in activity occurring in November. A major takeaway is the characterization of established populations in multiple Kentucky counties that have no previous record of this tick. *Ixodes scapularis* appears to be well-established in Kentucky but with a more restricted distribution and lesser abundance compared to *A. americanum* and *D. variabilis.* This tick may be active any month of the year and caution should be taken year-round to mitigate exposure. Finally, the detection of *B. burgdorferi* in unfed, host-seeking blacklegged ticks from Kentucky provides compelling scientific evidence for the expansion of Lyme disease into the southeastern United States.

The primary objective of this dissertation research was to provide a large-scale, multi-year dataset describing tick and tick-borne pathogen distributions in Kentucky between the years of 2019 to 2022. Going forward, there are many avenues on which to base future studies, including efforts to investigate control measures, target the invasive Asian longhorned tick, or perform more structured surveillance to determine tick and pathogen densities.

The immediate next steps should focus on performing more robust surveillance in agriculture-specific environments to determine the prevalence of tick-borne pathogens in host-seeking tick populations and to perform field trials to investigate the efficacy of control measures in different environments. Studies suggest that the occupational risk of tick-borne disease is 3-10 times higher for outdoor workers (Smith et al., 1988; Schwartz & Goldstein, 1990), but efforts to develop realistic approaches to prevent tick-borne disease in agricultural communities have been few, leaving many to simply accept these risks as part of the job. Agriculture and forestry play crucial roles in Kentucky's economy, thus investing in the health of Kentucky's farmers, forestry workers, and livestock is crucial to ensure the continued success and prosperity of these vital industries.

Persons working in agriculture and forestry are at an exceptionally greater risk for tick bite exposure (Smith et al., 1988; Schwartz & Goldstein, 1990). For several reasons, research aimed at controlling ticks and tick-borne diseases in agriculture landscape is important. Ticks are a major public health concern, posing a significant threat to rural areas where people are more likely to encounter infected ticks, and are also major veterinary pests causing considerable morbidity and mortality in livestock and companion animals. In Kentucky, the cattle industry is one of the largest in the nation and generates around $45.6 billion annually for the state (USDA, 2019), while the Kentucky Forest Sector provided an estimated 50,000 jobs and contributed $13.18 billion to the economy in 2021 (Kentucky Master Logger Database). Additionally, Kentucky's world-renowned reputation as the horse capital of the world, with its rich history of horse breeding, famous equine farms, and major racing events, contributes billions of dollars and thousands of jobs each year (Coleman et al., 2015). Tick-borne disease or other adverse effects from tick exposure

would lead to decreased productivity, reduced working efficiency, and increased control costs, resulting in substantial losses for these industries and the state. Additionally, surveying agriculture-intense areas would likely yield detections of the invasive *H. longicornis*, as it is known to frequent and pose major threats to livestock.

Future studies focused on this topic could utilize the following framework: 1) Perform targeted surveillance for key tick species and pathogens on farms, 2) Work with appropriate organizations and researchers to develop reliable pest control and disease prevention approaches, and 3) Develop training modules for county agricultural agents, health departments, and educators on arthropod vectors and vector-borne disease. Outreach programs are essential in preventing and managing tick-borne diseases. Tick-borne diseases are often underdiagnosed and underreported, which leads to a lack of awareness and understanding among the public. By including outreach programs, researchers can combat this issue by promoting best practices for preventing tick-borne diseases including personal protective measures, and the safe and effective use of chemical and non-chemical tick control methods. To maximize the impact of outreach efforts, collaboration with park and recreation departments, schools, community organizations (FFA, Cattleman's Association), and health departments should be included to share information about tick-borne diseases, their vectors, and findings of research. UK's College of Agriculture could serve as a pathway in which to connect directly with agricultural workers to discuss the risks they face and provide information on how they can work to best protect themselves and decrease the risk of tick-borne diseases.

The implementation of a comprehensive education program, including in-person workshops and online seminars, would target a diverse group of professionals seeking

training in vectors, vector-borne diseases, and the larger field of public health entomology. By using a variety of channels, such as social media, radio, and newsletters, one could reach a broader audience. These outreach efforts should emphasize low-cost, low-effort control measures, such as personal protective measures (PPMs), and provide information on the proper use of repellents and acaricides, while also encouraging workers to participate and provide feedback on the ease of use and effectiveness of these methods and emphasize the importance of seeking prompt medical attention for tick bites and tick-borne illnesses to ensure timely diagnosis and treatment.

I also collected 232 *H. longicornis,* 28 *D. albipictus*, 11 *H. leporispalustris*, one *I. brunneus,* and one *R. sanguineus* tick. In total, I collected data for 119 of Kentucky's counties, leaving only one county without data: Fulton County. These findings highlight the need for increased research and development of a sustainable surveillance program to monitor tick populations and associated diseases in the state. Between the tick species described here, tick season appears to be every season. As such, strategies to prevent exposure should be implemented year-round. In conclusion, this dissertation provides valuable insights into the distribution, abundance, and seasonality of tick vectors and associated pathogens in Kentucky, with particular emphasis on the dynamics of Lyme disease, ehrlichiosis, and spotted fever rickettsioses and their respective tick vectors. The research is of great use for vector control, public health, and veterinary health fields, and provides a valuable framework for future studies to address the growing concerns of tick and tick-borne diseases.

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PROFESSIONAL POSITIONS

2019 -2023 Graduate Research Assistant, University of Kentucky

2020-2023 Graduate Teaching Assistant, University of Kentucky

2021-2023 Graduate Student Officer – Secretary

2021-2022 Graduate Student Officer – Social Chair

2019-2021 Nursery Inspector, State Entomology Office, University of Kentucky

AWARDS, HONORS, & RECOGNITIONS

2020-2023 Central Appalachian Regional Education & Research Center Fellow

2022 SECVBD Dodd Short Courses Fellows

2022 1ST Place ESA Graduate Student Infographic

2021 1ST Place ESA Graduate Student Virtual 10-Minute Paper in MUVE

2021 Southeastern Conference (SEC) Emerging Scholar

2020 2nd Place ESA Graduate Student Poster in MUVE

2020 UK CAFE Graduate Student Spotlight

PUBLICATIONS

Pasternak, A. R. (2021). "Kentucky Veterinarian Tick Submission Program: Surveillance, Speciation, & Diagnostics." Proceedings of the Kentucky Veterinary Medical Association meeting. Louisville, Kentucky. Sept. 24-26.

Pasternak, A. R., & Palli, S. R. (2022). Mapping distributions of the Lyme disease vector, Ixodes scapularis, and spirochete, Borrelia burgdorferi, in Kentucky using passive and active surveillance. Ticks and Tick-borne Diseases, 101885.

Pasternak, A. R., & Palli, S. R. (2023). County-level surveillance for the lone star tick, Amblyomma americanum, and its associated pathogen, Ehrlichia chaffeensis, in Kentucky. Ticks and Tick-borne Diseases, 14(1), 102072**.**

Pasternak, A. & Larson, J. (2021). "ENTfact 518: Preparing for Asian Longhorned Tick in Kentucky". Fact Sheet. University of Kentucky. https://entomology.ca.uky.edu/ef518

Pasternak, A., Larson, J., & Cipriani, M. (2021). "ENTfact 618: Ticks and Disease in Kentucky." Fact sheet. University of Kentucky. https://entomology.ca.uky.edu/ef618

Larson, J., & **Pasternak, A**. (2020). "Blacklegged Ticks Keep on Ticking Regardless of Winter". Kentucky Pest News.

Submitted Manuscripts

Pasternak, A. R. & Palli, S. **"**Distribution of Dermacentor variabilis and Amblyomma maculatum (Ixodida: Ixodidae) and two spotted fever group rickettsia pathogens in Kentucky. Submitted to the Journal of Medical Entomology.