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DOCUMENTING RANGE EXPANSION OF TWO INVASIVE TICK (ACARI:IXODIDAE) SPECIES IN EASTERN SOUTH DAKOTA

Braden Wojahn
University Of South Dakota

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By

Braden Wojahn

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satisfactory and recommend that it be accepted.

Hugh Britten

Chairperson Hugh Britten Ph.D.

Daniel Soluk

Daniel Soluk Ph.D.

Jeff Wesner Ph.D.

ABSTRACT

This project documents the expanded zoogeographic ranges of the blacklegged tick (*Ixodes scapularis*) and lone star tick (*Amblyomma americanum*) within eastern South Dakota. Ticks are important when considering a "One Health" approach, as many species are competent vectors of zoonotic pathogens causing human diseases and conditions such as Lyme disease, tularemia, rickettsia, and alpha-gal syndrome. The research involved constitutes two steps. First, tick surveillance was conducted in eastern counties of South Dakota, spanning March 2021 through August 2022. Surveillance took place along edge habitat at recreation areas, state and public parks, wildlife management areas and refuges, and other natural areas that could be identified as suitable tick habitat. All life stages and species of ticks were collected and preserved in microcentrifuge tubes filled with 95% ethanol and placed in a freezer at -20 deg C. All target species of ticks were collected using a white cloth tick drag. Second, collection data were analyzed and compared to collection data from similar tick sampling efforts from 2019 and 2020 to determine if, and to what extent, range expansion had occurred. Distribution maps were created for each year to represent areas where *I. scapularis* and *A. americanum* were reported, and changes in species establishment were displayed. This study focused primarily on the Southeastern region of the state, near where *I. scapularis* populations exist in bordering Minnesota, and *A. americanum* in Nebraska and Iowa. As of the 2022 sampling season, the only counties where *I. scapularis* and *A. americanum* appear to have overlapping ranges are Clay County and Union County. *Ixodes scapularis* and *A. americanum* both have overlapping habitat with the more widespread and common established species *Dermacentor variabilis*, the American dog tick. This study also yielded a state record for another human disease vector, the gulf coast tick (*Amblyomma maculatum*) in 2021 and 2022. A weak positive correlation was calculated between total ticks collected and the person-hours spent to collect those ticks. Target species of tick were detected in nine counties in eastern South Dakota.

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Dr. Hugh Britten

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Chapter 1: Introduction

Background

General tick information

Hard-backed ticks (Ixodidae) are parasitic haematophagous arachnids that have a worldwide distribution with over 700 species currently described (Guglielmone et al. 2010). The Ixodidae life cycle consists of egg, larval, nymphal, and adult stages. Adult female ticks may lay several thousand eggs, although this number varies by species (Drummond et al. 1971). Ixodidae differ from the soft ticks (Argasidae) morphologically as the Ixodid ticks possess a scutum, and the gnathosoma is projected outward from the cephalothorax rather than tucked underneath. Hard-backed ticks feed on the blood of vertebrates by inserting their jagged mouthparts (hypostome) beneath the skin of their host. They inject salival compounds that contain anesthetics, making it more difficult for the host to discover the feeding tick. They can remain on a host for many days and may have more than one host, depending on the species. Some species must feed on a different host at each life stage, while others are generalists. Tick feeding can promote the transmission of pathogens and parasites, with ticks serving as a vector between reservoir hosts (hosts that contain the pathogen but do not exhibit disease) and primary hosts (where the pathogen or parasite reaches maturity). If a reservoir host has a bloodborne infection, the feeding tick can ingest that particular pathogen and subsequently introduce it to a new host when they feed again. For this reason, ticks are of significant public health importance and are a common focus of disease ecology.

Ticks generally seek out vertebrate hosts by ascending vegetation and holding on to it with their 3rd and 4th pairs of legs; this behavior is referred to as “questing” (Leal et al. 2020). When a potential host passes an area where ticks are questing, the ticks can attach to the host using specialized leg morphologies. Questing duration and frequency is influenced by a variety of factors, which indirectly impacts disease transmission to host organisms. Larval ticks quest after hatching and can obtain pathogens from the host animal they feed upon. *Ixodes* spp. nymphs, for example, normally spend their life within leaf litter, but will begin to quest when the next blood meal is required, which is a critical time for the survival of both the nymph and the Lyme Disease spirochete bacterium *Borrelia burgdorferi* (McClure & Diuk-Wasser 2019). Ticks need to balance time spent questing with water loss, as ticks that vertically ascend vegetation are at risk of desiccation and ultimately death (Nielebeck et al. 2023). If ticks begin to lose water too rapidly as they wait for a host, they descend the vegetation and wait closer to the ground where they attempt to rehydrate. Ticks can imbibe moisture from surrounding air, but the need to retain moisture is more important than the ability to regain it (Stafford, 1994). As such, tick survivability can be largely influenced by water regulation characteristics such as humidity (Nielebeck et al. 2023).

Multiple hypothesized invasion patterns for ticks and their associated pathogens were postulated by Hamer and others (2010) to describe temporal and spatial aspects during the early stages of invasion. “Tick-first” invasion occurs when uninfected ticks are transported to new areas, usually by a non-susceptible host. The pathogen eventually becomes present in the newly established ticks through secondary invasion via susceptible hosts and/or infected ticks. Another scenario is “dual invasion,” whereby ticks arrive in new areas via avian or mammalian hosts concurrently with the pathogen. If the pathogen is detected in the tick population early in the invasion when tick densities are low, dual invasion has likely occurred. One other potential scenario, “pathogen-first,” involves the pathogen being present first before invasion of tick vectors, usually harbored within reservoir hosts and cryptic vector species. Upon arrival of ticks, the pathogen can then be transmitted to a broader range of species than prior to invasion. Due to the complexity of disease ecology paired with range expansion, other invasion types may exist but have not been hypothesized or observed.

Ticks and disease

In the contiguous United States, only a small number of tick species regularly come into contact with and transmit pathogens to humans (CDC 2019). Although few ticks are capable of causing human diseases, the variety of pathogens they can transmit are greater than any other blood-feeding arthropod (Sonenshine 2018). Ticks are a public health concern because many of the diseases they are associated with can be debilitating and life-threatening. Recent attention has been drawn to the increasing ranges of the main vector species in the US, and questions have arisen about the implications of expanding tick ranges for public health (Sonenshine 2018). The effects of climate change and human alterations of habitat are likely allowing ticks to survive in novel environments, although other factors such as host availability/specificity and humidity tolerance are not fully understood (Sonenshine 2018).

Many tick species are important organisms for the study of disease ecology as they are vectors of pathogens and parasites that afflict vertebrates. As vector populations extend their range into previously uninhabited areas, the pathogens associated with these species often disperse with them (Westerdahl et al. 2014), meaning higher incidence of tick-borne illness in some regions compared to prior years. This can become problematic as knowledge of newly introduced tick-borne illnesses may not be accessible to people until after the effects or disease become apparent. Factors leading to expansion of ticks in the United States include climate change (Sonenshine 2018), land use and habitat alterations (Diuk-Wasser et al. 2020), and host interactions (Tsao et al. 2021).

Ticks and the One Health Concept

Being aware of the diversity of tick species, range and habitat information, and the pathogens associated with each species is a vital step in developing the "One Health" approach, with the aim of attaining optimal health of ecosystems. One Health is a collaborative, multidisciplinary approach that takes into account the relationships between humans, other animals, plants, and the environment (CDC 2018). This concept has become increasingly important in recent years, with more connections being made between disease ecology and human/animal health (Cunningham et al. 2017). It suggests that the overall health of ecosystems correlates with the health of humans and domesticated animals, and that humans have reduced the overall health of ecosystems through actions such as habitat destruction and the exacerbation of climate change. These types of changes can increase the prevalence of emerging infectious diseases, many of which are zoonotic in origin, such as tick-borne pathogens. As tick populations move into regions where they historically have not existed, the pathogens they carry are also introduced to the system. Knowledge of tick range expansion can inform not only biologists, but also medical professionals, veterinarians, relevant government agencies, and the public. Medical professionals may not be fully informed of the extent to which novel tick-borne pathogens are entering the regions they service, and may fail to correctly diagnose and treat tick-borne illnesses they would not expect to encounter. Routine tick surveillance is vital to understanding not only which tick species are present, but also the pathogens that may pose a risk to the public.

South Dakota as a habitat for ticks

South Dakota is located in the upper midwestern United States in the Great Plains region. Around four percent of land in South Dakota can be considered woodland habitat (Walters 2016), much of which consists of riparian areas and the Black Hills National Forest. Much of the remaining land is used for agriculture, and thus has experienced immense change which has impacted many “neutral” ecosystems in the state. The ability to occupy recently disturbed areas is a common characteristic of invasive species (Meyer et al. 2021), and therefore certain areas within the state may be subject to invasion by tick species expanding their ranges from states bordering South Dakota (Black et al. 2021).

Trophic model and tick-borne illness

In a trophic cascade model, predator numbers can directly and indirectly impact lower trophic levels (Preisser, 2007). With fewer predators present, the prey species of those predators and in turn the pathogens and parasites associated with those prey species may experience increased populations. For example, the white-footed mouse (*Peromyscus leucopus*) has been demonstrated to be a competent reservoir host of *B. burgdorferi* (Donahue et al. 1987) and using a trophic model it would be expected that there would be higher prevalence of *B. burgdorferi* when predator populations of the white-footed mouse are low. In eastern states, abundance of coyotes (*Canis latrans*) has resulted in lower numbers of red fox (*Vulpes vulpes*) and consequently an increase in abundance of small mammal hosts of *Ixodes* species, especially *P. leucopus* (Levi et al. 2012). When *C. latrans* preys on *V. vulpes* enough to significantly decrease their population numbers, *V. vulpes* predation on *P. leucopus* is also decreased, potentially leading to higher population numbers of *P. leucopus*. If this is the case, significantly more feeding can be achieved by *I. scapularis* on *P. leucopus* and additionally a higher rate of *B. burgdorferi* in the tick populations. In this respect, small-mammal predator population numbers can somewhat serve as a predictor for Lyme Disease prevalence in humans (Levi et al. 2012).

Additionally, ecosystems with higher vertebrate diversity and a variety of species serving as reservoir hosts for *B. burgdorferi* have a lower vector infection prevalence (individuals with disease at a given point in time) (LoGiudice et al. 2003); this phenomenon is known as the dilution effect. Species-poor ecosystems may contain higher numbers of *P. leucopus* but fewer predators and alternative host species for *Ixodes* spp., meaning that ticks would be feeding heavily on *P. leucopus* as a commonly available host. Because blacklegged ticks are generalist feeders, species-rich ecosystems should result in lower transmission of the pathogen from white-footed mice to feeding *Ixodes* vectors (LoGiudice et al. 2003). This illustrates one instance of biodiversity and species richness being linked to the health of an ecosystem, as emphasized by the One Health concept.

Ticks in South Dakota

There are 21 tick species found in South Dakota, with four of these species considered common competent vectors of pathogens that affect humans: *Amblyomma americanum, Dermacentor variabilis, Dermacentor andersoni,* and *Ixodes scapularis* (Maestas 2019). These species are capable of transmitting a diversity of pathogens that affect humans such as *Borrelia* spp., *Babesia microti*, *Anaplasma phagocytophilum*, *Ehrlichia* spp., *Francisella tularensis*, and *Rickettsia* spp. among others (CDC 2023). There are multiple other conditions associated with tick bites, like alpha-gal syndrome and tick paralysis, which are not well understood (CDC 2023). Tick range data is historically lacking in South Dakota, and recent distribution maps may not accurately represent the true presence of species within the state. Routine surveillance efforts and collaboration with local biologists are often required to determine where tick populations occur and to understand the ecology of each species. Recent incidental reports of important vector species within eastern counties of South Dakota have led to increased interest in tick diversity within the state. Based on these studies I decided to focus on two species, the blacklegged tick (*Ixodes scapularis*) and the lone star tick (*Amblyomma americanum*), both of which are vector-competent for human pathogens.

The black-legged tick (*Ixodes scapularis*) is the primary vector of *B. burgdorferi*, the causative agent of Lyme borreliosis, in eastern and midwestern states. This species prefers wooded habitat where the immature stages (larvae and nymphs) overwinter in leaf litter (Schulze and Jordan 2006). Regions characterized by low leaf litter quantity and harsh winter temperatures may result in higher mortality of *I. scapularis* (Linske et al. 2019). There are few habitat patches in eastern South Dakota that currently support populations of *I. scapularis* and others that could be considered suitable but are not known to support populations (Maestas et al. 2016, 2018, Black et al. 2021). It has been suggested that climate warming trends may increase the survival and geographic range of *I. scapularis* and their host species (Linske et al. 2019). While scattered cases of *I. scapularis* have been reported from hosts in South Dakota for many years (McDaniel and Hildreth 1992), collections of host-seeking *I. scapularis* were only confirmed following an incidental report in 2015 (Maestas et al. 2016). Subsequent surveillance efforts found evidence of established populations in Clay County, near a riparian backwater habitat along the Missouri River (Maestas et al. 2016) and across eastern South Dakota in four additional counties (Maestas et al. 2018). These reports did not meet the establishment criteria of Dennis et al. (1998) and the CDC.

All *Ixodes* spp. collected during the 2015-2017 sampling seasons were tested for presence of *B. burgdorferi* using quantitative polymerase chain reaction (qPCR) methods. Of these, only one individual collected from Sica Hollow State Park (Marshall County) was *B. burgdorferi*-positive (Maestas et al. 2018). Testing of ticks collected during the 2019 sampling season revealed *B. burgdorferi* from two additional counties (Day County, Lincoln County) (Black et al.

2021). Evidence of *B. burgdorferi* in host-seeking *I. scapularis* in the state, in addition to the multi-county reports, suggest a higher likelihood of future instances of Lyme Disease in eastern South Dakota. Previous studies such as one by Hamer et al. (2010) have documented expansion of *I. scapularis* in real-time over a relatively short time frame (five years) which may serve as a predictor of expansion and tick-borne illness trends for *I. scapularis* in states such as South Dakota. Due to the confirmed presence of *B. burgdorferi* in low-density populations of *I. scapularis*, I suspect that these species were introduced to the state in a "dual invasion," assuming we are in the early stages of invasion as the historical evidence would suggest.

The lone star tick (*Amblyomma americanum*) has habitat preferences similar to that of *I. scapularis*, although it prefers edge habitat near wooded areas and does not necessarily require leaf litter in its life cycle (Springer et al. 2015). This species has historically been absent from South Dakota, with the exception of isolated instances of *A. americanum* being collected from animal hosts (Springer et al. 2014, Maestas 2019). With the goal of addressing the lack of tick range data in the state and further investigating the expansion of *I. scapularis,* surveillance efforts were conducted in Spring and Summer of 2019 and 2020 by Black et al. (2021) in eastern South Dakota during months when ticks are actively questing. They found the first evidence of host-seeking populations of *A. americanum* in the state, including multiple established populations (Black et al. 2021). Nebraska and Iowa both harbor established populations of *A. americanum* (Raghavan et al. 2019), suggesting these populations have spread northward and remained undetected due to a lack of surveillance. Black et al. (2021) is currently the only comprehensive study of *A. americanum* populations in the state, and therefore scope of tick-borne illness associated with this species is not yet well known. As such, we found it necessary to continue this work to learn additional information about the status of the lone star tick in eastern South Dakota.

Historically absent from South Dakota, both *I. scapularis* and *A. americanum* now have established populations in more than one county in the eastern part of the state. Prior reports of these species being collected from mammalian hosts (McDaniel and Hildreth 1992, Springer et al. 2014) did not meet criteria for establishment, and incidental host data is not always reliable for determining existence of populations due to long-distance movement of host species. The presence of *I. scapularis* and *A. americanum* in South Dakota may lead to increased tick-borne illness in wildlife, livestock, and humans, so determining the extent of vector range expansion is critical. This thesis aims to combine past surveillance data with current data to provide insight into the pattern of range expansion of *I. scapularis* and *A. americanum* to inform public health practices in South Dakota.

Chapter 2: Range Expansion

Abstract

Many tick species are important vectors of zoonotic pathogens causing human diseases and conditions such as Lyme disease, tularemia, rickettsia, and alpha-gal syndrome. Knowledge of tick diversity and distribution is vital in a “One Health” approach, to better understand and maintain health of ecosystems. This project documents the expanded zoogeographic ranges of the blacklegged tick (*Ixodes scapularis*) and lone star tick (*Amblyomma americanum*) within eastern South Dakota. The research required two steps. First, tick surveillance was conducted in eastern counties of South Dakota using a white cloth tick drag from March 2021 through August 2022. Surveillance took place along edge habitat at recreation areas, public parks, wildlife management areas, and any other natural areas that could be identified as suitable tick habitat. All life stages and species of ticks were collected and preserved in microcentrifuge tubes filled with 95% ethanol and placed in a freezer at -20 deg C. Second, collection data were analyzed and compared to collection data from similar tick sampling efforts from 2019 and 2020 to determine if, and extent of range expansion. Distribution maps were created for each year to represent areas where *I. scapularis* and *A. americanum* were reported, and changes in species establishment were displayed. As of the 2022 sampling season, the only counties where *I. scapularis* and *A. americanum* appear to have overlapping ranges are Clay and Union Counties. These species both partially share geographic distributions with the more common and widespread established species *Dermacentor variabilis*. This study also yielded a potential state record for another human disease vector, the gulf coast tick (*Amblyomma maculatum*) in 2021 and 2022. This study indicates that ticks are an emerging public health threat in eastern South Dakota, and that both *I. scapularis and A. americanum* are established in the state.

Introduction

Hard-backed ticks (Ixodidae) are parasitic haematophagous arachnids with a worldwide distribution, and over 700 species are currently described (Guglielmone 2010). Certain genera are known to be prominent vectors of emerging pathogens affecting public health in the United States, mainly *Ixodes*, *Amblyomma*, and *Dermacentor* (Rochlin and Toledo, 2020). Lyme Disease accounts for the majority of vector-borne disease in the country, with some 300,000 cases reported annually (Nelson et al. 2015). Other tick associated diseases are less frequently diagnosed or reported, but actual numbers of cases may be much higher (Rochlin and Toledo, 2020).

In the contiguous United States, only a small number of tick species regularly come into contact with and transmit pathogens to humans (CDC 2019). Although few ticks are capable of causing human diseases, the variety of pathogens they can transmit are greater than any other blood-feeding arthropod (Sonenshine 2018). Ticks are a public health concern as many of the diseases they are associated with can be debilitating and even life-threatening. Recent attention has been drawn to the increasing ranges of the main vector species in the US, and questions have arisen about the implications of expanding tick ranges to public health (Sonenshine 2018). The effects of climate change and human alterations of habitat are likely allowing ticks to survive in novel environments, although other factors such as host availability/specificity and humidity tolerance are not fully understood (Sonenshine 2018).

Tick range information can be useful for informing public health efforts and regionally diagnosing tick-borne illnesses. As vector populations extend their range into previously uninhabited areas, the pathogens associated with these species often disperse with them (Westerdahl et al. 2014). This means that certain regions may experience higher incidence of tick-borne illness compared to prior years. Knowledge of newly introduced tick-borne pathogens may not be accessible to people until after the effects or disease become apparent. Being aware of the diversity of tick species, range and habitat information, and the pathogens associated with each species is a vital step in developing "One Health," with the aim of attaining optimal health of ecosystems (CDC 2018).

Concern over range expansion of many of these species has prompted numerous studies into tick ecology and disease dynamics, e.g., Hamer et al. (2010); Springer et al. (2014, 2015); Maestas et al. (2016, 2018); Black et al. (2021). Prior studies in eastern South Dakota provided new evidence for established populations of *Ixodes scapularis* and their associated pathogens (Maestas et al. 2016; Maestas et al. 2018;) and the first reports of host-seeking *Amblyomma americanum* (Black et al. 2021). Surveillance for host-seeking ticks in South Dakota has been lacking until recent years, and the results from the aforementioned studies suggest an increased need for determining current vector ranges.

South Dakota is located in the upper midwestern United States in the Great Plains region. Only around four percent of land in South Dakota can be considered woodland habitat (Walters 2016), making the state less suitable for many tick species. Much of the land within South Dakota is used for agriculture, and thus has experienced immense change which has implications for the ecosystems in the state. Habitat conversion may increase the potential for contact between ticks and humans, as ticks might currently exist or move into areas that have been developed for human use.

Tick range data are historically lacking in South Dakota, and recent distribution maps may not accurately represent the presence of species within the state. Routine surveillance efforts and collaboration with local biologists are often required to determine where tick populations occur and to understand the ecology of each species. Recent incidental reports of important vector species within eastern counties of South Dakota led to increased interest in tick diversity within the state. Prior studies involving various tick monitoring methods have shed light into the tick species diversity in South Dakota as well as new zoogeographic range data for multiple species, e.g., Maestas et al. (2016, 2018); Maestas (2019); Black et al. (2021).

This study will focus on determining the current ranges and potential expansion of the black-legged tick (*Ixodes scapularis*) and the lone star tick (*Amblyomma americanum*) in eastern South Dakota. Criteria used to distinguish between presence and establishment are based on work by Dennis et al. (1998) and the CDC. A tick species is considered established in an area if either of the following are fulfilled; six or more ticks of that species are collected within a sample period, or more than one life stage of that species is collected within a sample period. If fewer than two life stages or fewer than 6 individuals of a species were collected within a sampling period, the species would be considered present in the location but not established. A sample period consists of the earliest through the latest surveillance outing within a calendar year. Establishment in a given area can be represented minimally at the site level and more broadly at the county level.

The black-legged tick (*I. scapularis*) is the primary vector of *Borrelia burgdorferi*, the causative agent of Lyme Disease, in eastern and midwestern states. This species generally prefers wooded habitat as the immatures overwinter in leaf litter, and they may experience higher mortality in regions characterized by low leaf litter quantity and harsh winter temperatures (Linske et al. 2019). Due to limited woodland habitat, the majority of land in the state is not suitable for *I. scapularis* (Maestas et al. 2018). There are few habitat patches in eastern South Dakota that currently support populations of *I. scapularis* and others that could be considered suitable but are not known to support populations (Black et al. 2021). It is suggested that climate warming trends may increase the survival and geographic range of *I. scapularis* and their host species (Linske et al. 2019). Past attempts to detect *I. scapularis* populations in bordering Minnesota have occurred primarily in the 21st century by the Minnesota Department of Health, which represents significant effort into determining presence of the species. Tick surveillance data were compiled and used to model habitat suitability for *I. scapularis* in the state (Johnson et al. 2016). Recent surveillance for *I. scapularis* and other species in Minnesota has primarily focused on pathogen prevalence rather than establishment and expansion (Johnson et al. 2018, Whitten et al. 2019).

Ixodes scapularis is classified as established in 35 states of the contiguous United States, with most established counties occurring in the northeastern and northern-central states (Eisen et al. 2016). Over the past two decades, the total number of counties with established populations have doubled, suggesting geographic expansion of *I. scapularis* in all cardinal directions (Eisen et al. 2016). *Ixodes scapularis* was previously reported from two counties in South Dakota (Brookings and Codington), but established populations were not confirmed (Dennis et al. 1998). Surveillance efforts by Maestas et al. (2016) provided evidence for at least one established population in Clay County as early as 2015, at a riparian backwater habitat along the Missouri River. Further surveillance across eastern South Dakota revealed four additional counties with *I. scapularis* present, but these findings did not meet the establishment criteria (Maestes et al. 2018).

All *I. scapularis* collected during the 2015-2017 sampling periods were screened for presence of *B. burgdorferi* using genomic DNA extraction and quantitative PCR methods (Maestas et al. 2018). Of these, only one individual collected from Sica Hollow State Park

(Marshall County) was *B. burgdorferi*-positive (Maestas et al. 2018). Two ticks collected in 2019 were *B. burgdorferi*-positive; one each from Day and Lincoln counties (Black et al. 2019). Evidence of *B. burgdorferi* in host-seeking ticks in the state, in addition to the multi-county reports of *I. scapularis*, suggest an increasing likelihood of future instances of Lyme Disease in eastern South Dakota.

The lone star tick has habitat preferences similar to that of *I. scapularis*, although it prefers edge habitat near wooded areas and does not necessarily require leaf litter in its life cycle (Springer et al. 2015). *A. americanum* has historically been absent from South Dakota, minus isolated instances of ticks collected from animal hosts (Springer et al. 2014). With the goal of addressing the lack of tick range data in the state and further investigating the expansion of *I. scapularis*, Black et al. (2021) surveyed eastern South Dakota during months when ticks are actively questing and found established host-seeking populations of *A. americanum*. Established populations occur in Nebraska and Iowa (Raghavan et al. 2019), suggesting the newly discovered established populations have spread northward from these regions and remained undetected due to a lack of surveillance. Black et al. (2021) is currently the only comprehensive study of *A. americanum* populations in the state, and therefore the scope of tick-borne illness associated with this species is not yet well known. As such, we found it necessary to continue this work to learn additional information about the status of the lone star tick in eastern South Dakota.

The focal species of this study are recently confirmed from South Dakota through rigorous surveillance efforts. Historically absent from South Dakota, both species now have established populations in more than one county in the eastern part of the state. The presence of these species in South Dakota may lead to increased tick-borne illness in wildlife, livestock, and humans. Absence of tick surveillance data may account for misdiagnoses of diseases associated with these species, so determining the extent of vector range expansion is critical. This study combines past tick surveillance research (Maestas et al. 2016, Maestas et al. 2018, Black et al. 2021) with additional data to provide insight into the range expansion of the focal species of this study and inform public health in South Dakota.

Methods

Tick Surveillance

Tick surveillance occurred in Spring and Summer of 2019 through 2022 in eastern South Dakota. Sampling seasons were characterized as the first "surveillance instance" through the last "surveillance instance" within a single year. Each sampling season started early Spring and concluded in early Autumn, with variable start and end dates between years. Surveillance occurred along edge habitat at recreation areas, public parks, wildlife management areas, and any other natural areas that could be identified as suitable tick habitat. Effort was focused in the eastern counties of South Dakota; no surveillance was conducted west of Charles Mix County.

Sampling was conducted using the "tick drag method," one of the most common and effective techniques for collection of host-seeking ticks (Salomon et al. 2020). Tick drag flags were constructed by securing a $1m^2$ piece of white cotton fabric with a sleeve onto a 1.5-meter wooden dowel. A binder clip was used to prevent the fabric from slipping off the dowel during surveillance. To collect questing ticks, the flag was dragged across edge habitat vegetation at a regular walking pace. Periodically, the flag was checked for presence of ticks on the fabric, generally every 10-50 meters. If any number of *I. scapularis* or *A. americanum* were collected along a stretch of habitat, that same length was surveyed again to obtain any ticks that may have been missed. In sites where no ticks were collected, surveillance concluded after 1 person-hour of effort. In sites where ticks were collected within the first person-hour, surveillance concluded after 1 person-hour of effort following the last tick that was collected. Ticks were removed from the fabric with dissecting forceps and transferred to microcentrifuge tubes (1.5 mL) filled with 95% ethanol. For every surveillance site, sampling effort was timed and recorded. Environmental factors such as temperature, humidity, and habitat type were recorded for each site where ticks were collected. Tick specimens were identified to species, life stage, and sex when possible. All life stages and species of tick were preserved in the microcentrifuge tubes and placed in a freezer at -20 °C for future reference.

Data Analysis

Surveillance effort was expressed as person-hours to account for the variable number of personnel sampling at each location. Total numbers of each tick species and total person-hours were summed for every surveillance year. Linear regressions were used to express the relationships between ticks collected versus the person-hours spent to collect them. Plots and regressions were expanded by separating total ticks by species and person-hours by surveillance year. A representation of all surveillance instances was created to compare species presence between surveillance sites. Only sites and surveillance instances where at least one tick was collected were represented in our analysis. Larval collections of over 75 individuals in one surveillance instance were omitted from all plots to avoid inflating the values of total ticks. All plotted figures were created in RStudio programming software (R Core Team, 2019). Packages used included “readr” (Wickam et al. 2023), “tidyverse” (Wickam et al. 2019), “janitor” (Firke, 2023), “ggpmisc” (Aphalo, 2022), “ggpubr” (Kassambara, 2023).

Species-specific range maps were created for *A. americanum* and *I. scapularis* to visualize the extent of range expansion. All range maps were created using ArcGIS Pro mapping software. Counties with incidental encounters and established populations of *A. americanum* and *I. scapularis* were marked on a county map of eastern South Dakota. A species was considered to have an established population in a county when six individuals or more than one life stage of the particular species were found within a sampling period (Dennis et al. 1998). For example, if a larva and an adult of a given species were both collected in the same county within the sampling period, the county was considered to have an established population of that species. Collections of more than one life stage are indicative of successfully reproducing populations, and more than six individuals collected represents a density that would not be expected to occur if they were simply incidental introductions to the habitat. Establishment status in a county was retained year-to-year following a sampling period where establishment criteria were met, even if in a subsequent year establishment was not met for that county. Tick collection data for 2019-2020 were extracted from Black et al. (2021).

Results

Between 2019 and 2022, a total of 19 counties and 33 sites were sampled for questing ticks in eastern South Dakota with ticks found in 15 of these counties across 29 sites (Figure 1). The westernmost surveillance occurred in Charles Mix County, and the northernmost surveillance occurred in Roberts and Marshall Counties (Figure 1). Between all sampling sites and dates, four species of hard-backed ticks were collected using the tick drag method: *Amblyomma americanum, Amblyomma maculatum, Ixodes scapularis,* and *Dermacentor variabilis* (Figure 2). Total ticks collected of each species and life stage varied between years (Table 1). Person-hours also varied, with an average of 9.64 ticks collected per person-hour (Table 1).

Surveillance efforts revealed four counties in the state with sites that meet the establishment criteria for *I. scapularis*, and three counties with sites that meet the criteria for *A. americanum*. *Dermacentor variabilis* was collected from all sites where target species were collected, as well as many sites where target species were not collected. *Ixodes scapularis* and *A. americanum* were generally collected from different sites, with only three sites containing both species (Clay County Park, Gunderson Backwater, Union Grove State Park). Only Clay County was found to have established populations of both species. On multiple occasions, large numbers of *A. americanum* larvae were collected during single surveillance instances. Larval collections of over 75 individuals were not included in analysis to avoid skewing the dataset.

The Gulf Coast tick (*A. maculatum*) was collected from two counties in southeastern South Dakota across both surveillance years. A single male was found using the tick drag method near designated horse trails at Newton Hills State Park (Lincoln County) in June of 2021. It was collected along the entrance of Designated Horse Trail A, along the edge of the parking lot where park visitors frequently load and unload their horses. It was removed from the flag within the first few minutes of sampling, around 10 a.m. on June 15, 2021. After determining the identity of the specimen, an additional two hours were spent attempting to collect any other *A. maculatum* that may have been present at the site. A second individual (female) was collected from the body of the author during a non-surveillance outing at North Alabama Bend (Clay County) and was preserved using the same methods as described above.

Values of total ticks collected and the person-hours spent to collect those ticks are represented with a linear model and regression in Figure 3. Each data point represents a distinct surveillance instance consisting of a unique location and date, and all adult and nymphal ticks collected from that period are summed. The calculated R value for this relationship was 0.24, with a p value of 0.0011. The linear regression shows a slight positive correlation between the variables, with more sampling time resulting in higher numbers of ticks collected. The linear model was then expanded by species and person-hours by surveillance year (Figure 4). Only one of the individual species year-by-year regressions was significant (*D. variablis* in 2020) while one other *(D. variablis* in 2019) was marginally non-significant (Figure 4). R-values ranged from -0.23 (*Amblyoma* spp. in 2019) to 0.41 (*D. variablis* in 2020) (Figure 4). Surveillance instances were colored to represent the month in which the surveillance took place, showing the majority of ticks being collected between May and August.

Range maps for *I. scapularis* show year-by-year establishment from 2019 through 2022 (Figure 5), with counties remaining established between years even if establishment criteria were not met in a year following the confirmed establishment. Data from 2019 and 2020 were collected prior to this study by Black et al. (2021) and were incorporated for context of range expansion. By 2022, a total of four counties (Clay, Day, Lincoln, Union) met the requirements for establishment and two counties (Marshall, Roberts) had collections of *I. scapularis* that did not fulfill the requirements (Figure 5). The results of surveillance confirm that *I. scapularis* is established in the southeast and northeast regions of the state, in habitats containing dense forest patches. Range maps for *A. americanum* also show year-by-year establishment from 2019 through 2022 (Figure 6), with counties remaining established between years even if establishment criteria were not met in a year following the confirmed establishment. By 2022, a total of 3 counties (Clay, Union, Yankton) met the requirements for establishment and one county (Minnehaha) had a single report of *A. americanum*. Despite further sampling efforts, *A. americanum* was not found to be established or even present at any site in Minnehaha County, or any other county north of it.

Of the 29 sites sampled, all contained *D. variabilis*, seven contained *A. americanum*, and eight contained *I. scapularis* (Figure 7). The highest numbers of ticks were found at North Alabama Bend (Clay County), Newton Hills State Park (Lincoln County), Adams Homestead and State Nature Preserve (Union County), and Chalk Bluffs Multi-Use Trail (Yankton County).

Discussion

The surveillance efforts undertaken during the 2021 and 2022 sampling seasons revealed multiple established populations of *I. scapularis* and *A. americanum* in eastern South Dakota. The goal of the project was to maximize the counties and sites sampled to better understand the distribution of important vector species in the state. For this reason, counties that met the establishment criteria in one year may not have been revisited the following year as establishment was already determined. Notably, counties located between the southeastern and northeastern regions did not contain many ticks, with only *D. variabilis* present. This may be due to multiple factors such as the prevalence of agriculture, lack of suitable tick habitat, and inaccessibility of adequate surveillance sites. Many central-eastern counties contain little-to-no forested area and mainly consist of agricultural land, which is often hard to sample due to land ownership and inadequate areas to conduct surveillance. *Ixodes scapularis* may in fact be sparsely present in these counties, and it is doubtful that *Amblyomma americanum* can be found there.

Although they were collected previously in Clay County at neighboring Gunderson Backwater, our collections from 2021 revealed *I. scapularis* from North Alabama Bend, and in 2022 the species was considered established at this sampling location. *I. scapularis* was also abundant at Newton Hills State Park (Lincoln County) and Waubay National Wildlife Refuge (Day County), with fewer individuals collected from Union Grove State Park (Union County), Sica Hollow State Park (Marshall County), and Hartford Beach State Park (Roberts County). All sites where *I. scapularis* was collected consist of dense forest bordering grassland and prairie habitats, and contain significant leaf litter, all features consistent with *Ixodes* spp. ecology and lifecycle (Schulze and Jordan 2006). Very little of the land in South Dakota consists of forests, and therefore may not be suitable for many tick species, especially *I. scapularis*. Other forested sites were sampled but did not support *I. scapularis*, which may be explained by multiple factors. Certain sites that would otherwise be suitable for *Ixodes* spp. may have been sampled during times when adults are less active, or weather conditions (humidity, temperature) were not favorable for survival. Through the sampling periods of 2021-2022, much of South Dakota experienced extreme drought which may have significantly impacted the phenology of ticks during this time. Low humidity, low precipitation, and high temperature are not optimal for most ticks, and their host-seeking behavior is highly affected by these conditions (Leal et al. 2020).

In 2021, *A. americanum* was considered established at Gunderson Backwater (Clay County), which is coincidentally the same site where *I. scapularis* was first found to have established populations in the state. This site is made up of differing habitat types and is currently the only sampled site where established populations of both target species have been discovered. *I. scapularis* was first reported from Union Grove State Park in 2021 and was considered established the following year at this site. It is the only other sampling location where both target species have been collected, but only *I. scapularis* have established populations at this site. This is notable as the second-most northern occurrence of *A. americanum* in South Dakota (the first being the incidental encounter in Minnehaha County), which is roughly 24 km away from the nearest established population of *A. americanum* (Gunderson Backwater, Clay County).

When comparing the sites containing *A. americanum*, certain trends emerge. All sites are located in forested areas with ample edge habitat and varying foliage types and are situated near lotic ecosystems. White-tailed deer (*Odocoileus virginianus*) are a common host of all life stages of *A. americanum*, and the movement of deer along river corridors has been well documented (Nixon and Mankin 2011). With the abundance of white-tailed deer in South Dakota, the current distribution of *A. americanum* could be partially explained by the movement of white-tailed deer along forested riverine locations. This is consistent with our findings of established populations of *A. americanum* exclusively located along the Missouri River, in counties that share borders.

In multiple surveillance instances, large numbers of *A. americanum* larvae were collected in a very short period. Observations of clustered aggregations of larvae (referred to colloquially as "tick bombs") are common (Kennedy and Marshall 2021), and likely explain why hundreds of ticks would be present on the tick drag after sampling a short distance. Tick bombs occurred infrequently, generally in the later months of the sampling period. Adam's Homestead and Nature Preserve (Union County) was the only site where we observed this phenomenon. To avoid inflating the total tick values in all figures, surveillance instances where more than 75 larval *A. americanum* were collected were omitted. It is unclear whether *I. scapularis* clusters in this way, but larval numbers for this species were very low when they were detected by the tick drag method. For both species, presence of larvae was counted toward the requirements for establishment at a site as long as at least one other life stage was collected during the sampling period.

The movement of arthropod vector species has implications for One Health, and the results of this study demonstrate the need for increased awareness of commonly encountered host-seeking species of medical concern. Our updated range data should inform collaborative efforts to reduce tick borne illness in South Dakota and other susceptible regions. South Dakota residents frequently come into contact with *D. variabilis* as it is the most abundant species in the eastern counties. They may be unaware of the movements of prominent vector species to their region, and therefore may be less likely to take precautions that prevent tick-borne illness. Implementation of informative signage at public recreation areas and distribution of information from local health departments can be effective strategies for raising awareness to the public. Communication between public health departments and medical professionals can ensure that tick-borne illnesses are properly identified and less frequently misdiagnosed.

In addition to the target species of this study, *A. maculatum* was collected in both surveillance years from two different counties. To our knowledge, our 2021 collection of a single *A. maculatum* male is the first record of this species in South Dakota. Additionally, this specimen likely represents the most northwestern occurrence of *A. maculatum* in North America, although range maps for this species in the United States appear to be outdated and may not reflect the current distribution. Interestingly, the female collected in 2022 represents an incidence of the species that was not obtained using a conventional tick surveillance method. *A. maculatum* is not historically known from the upper Midwest and has only recently been detected in Nebraska (Nielsen et al. 2018) (Jeff Hamik, personal communication 2022). A few incidental reports of *A. maculatum* have been reported from Iowa, but there is no current evidence for established populations of this species in any states bordering South Dakota. In the most recent comprehensive review documenting the tick species of South Dakota, *A. maculatum* appears to be absent from surveillance data, and there were no reports of incidental occurrences of this species (Maestas 2019). Therefore, our collections of *A. maculatum* in South Dakota would bring the total number of tick species to 22.

A. maculatum was first collected during a normal tick surveillance outing at the entrance of a frequently used horse trail, using the tick drag method. The unexpected collection of this species prompted my speculation into the origin of the specimen in question. Adult Gulf Coast ticks generally feed on large mammals, including livestock and horses (Teel et al. 2010). Park patrons may travel from regions where *A. maculatum* have established populations, transporting their horses (and feeding ticks) to regions where the species is not generally found. With the high volume of horse traffic experienced at NHSP, it is possible that the adult male had previously fed on a horse before it was obtained using the tick drag. Out of 11 surveillance instances at this location in 2021, *A. maculatum* was only collected once and was not detected again in any sampling efforts following the initial discovery.

The second collection of *A. maculatum* was also unexpected, and prompted further questions into why the individual was found at the site. The female Gulf Coast tick was not collected using the tick drag method, rather it was collected from the body of the primary field technician (Wojahn, personal observation). The tick was found unattached climbing vertically up the ankle, implying it was likely searching for a host at the time of collection. The area in question was a semipermanent backwater of the Missouri River that was rapidly drying as a result of consecutive drought years. It is an open habitat with little protection from the sun and supports diverse species of emergent vegetation like grasses and shrubs, which provides ideal habitat for *A. maculatum* (Nadolny and Gaff 2018). Due to inaccessibility of the area and the absence of established population in the region, I find it unlikely that the individual collected from this site was transported by humans or companion animals. It has been postulated that the Gulf Coast tick can be transported into northern regions of North America via migratory birds along established flyways (Paddock and Goddard 2015; Sonenshine 2018), and 33 species of birds have been reported as hosts for immature *A. maculatum* (Teel et al. 2010). The Central and Mississippi flyways both cross eastern South Dakota so it is not out of the realm of possibility that bird species using these flyways could transport ticks long distances. This is one of the more likely explanations for the incidental *A. maculatum* collected in Clay County.

Multiple disease-causing pathogens of mammalian hosts are vectored by *A. maculatum*, including *Rickettsia parkeri*, *Ehrlichia chaffeensis*, *Hepatozoon americanum*, and others (Paddock and Goddard 2015). A condition of paralysis associated with feeding by *A. maculatum* has been reported several times in humans, but little is known about this association. Based on our findings regarding *A. maculatum*, it is reasonable to conclude that this species is not yet established in South Dakota, and therefore risk of the diseases associated with *A. maculatum* is low. Future surveillance efforts should be conducted in the southern counties of the state where *A. maculatum* would be more likely to invade, as well as in other regions where the range of this species is expanding. Surveys of birds that follow the two central flyways could reveal whether *A. maculatum* is transported via migratory birds to novel areas of the upper Midwest. Isolated established populations could occur if conditions are optimal, which may result in an increased public health risk as with the target species of this study. Multiple other states have also reported new records of *A. maculatum* within the last few years, (Nielsen et al. 2018, Phillips et al. 2020) suggesting that this species moves into new regions frequently. Another possibility is that *A. maculatum* does survive in these regions, but prior tick surveillance efforts were unable to confirm their presence.

The primary objective of the study was to determine locations in eastern South Dakota where established host-seeking populations of *I. scapularis* and *A. americanum* occur by conducting surveillance. When defining the region of "eastern South Dakota," there were no exact specifications in determining the boundaries for the region in question. South Dakota is often split into two distinct regions deemed “East River” and “West River” referring to the position of the Missouri River cutting North to South through the state. Surveillance occurred exclusively in the East River region, which we considered to be synonymous with eastern South Dakota.

To fit with the aforementioned research objectives, we decided that establishment criteria should only apply to host-seeking ticks identified from a single collection period (Dennis et al. 1998), which the United States CDC considers a 12-month period in which surveillance takes place. In prior studies, multiple tick collection types were used to determine establishment, including mammal trapping, bird netting, tick drag sampling, CO2 baiting, etc. (Dennis et al. 1998, Hamer et al. 2010). Although useful for investigating tick diversity, we did not use the methods of these studies because we specifically wanted to document actively host-seeking ticks to better understand the ranges of ticks commonly encountered by humans. Future studies in South Dakota to determine host species utilized by *I. scapularis* and *A. americanum* may provide insight into the specific mechanism(s) by which these species are expanding their ranges in the state.

Combining four years of data allowed us to visually represent total ticks collected in every surveillance instance plotted against person-hour time spent collecting those ticks (Figure 2). The outcome indicates a slight linear relationship between these variables, suggesting that more time spent at a site generally results in more ticks collected. Surveillance instances where more than 50 ticks were collected ranged from 0.5 to 5 person-hours, and sampling effort differed significantly between surveillance instances. The goal of our research was to determine locations where the focal species existed within our surveillance boundary (Figures 5 and 6), so it was counterintuitive to spend excessive time at sites where no ticks were collected after the first 0.5-1 hours of sampling. Studies with a more consistent sampling regime may find clearer results, as a focus can be made on understanding the optimal amount of time it would take to collect the largest number of ticks.

Chapter 3: Conclusions

This study aimed to follow previous studies by determining the extent to which range expansion of *I. scapularis* and *A. americanum* has occurred in eastern South Dakota. Tick sampling spanning 2019-2022 revealed previously unknown established populations of both *I. scapularis* and *A. americanum* as well as new reports of *A. maculatum*. Four counties in both northeastern and southeastern South Dakota were found to meet the criteria for establishment of *I. scapularis,* and *A. americanum* was found to be established in three southeastern counties. Sites where *I. scapularis* was collected were primarily woodland trails and edge habitat of forest patches that contained leaf litter. Sites where *A. americanum* was collected were primarily early successional habitats near or bordering the Missouri River. Neither species was collected in the central counties of eastern South Dakota, likely due to lack of suitable habitats. Additionally, these counties were surveyed less than in other regions due to inaccessibility of potential surveillance sites. It would be unsurprising if isolated populations of *I. scapularis* exist in these counties, and more effort focused on this region could provide a clearer answer. Based on our findings, it does not seem that *A. americanum* is established anywhere north of the three counties where established populations have been found. Future studies of tick range expansion in the Midwest would benefit from conducting surveillance near agroecosystems, where scattered populations may exist but remain undetected.

The Gulf Coast tick was collected from two different locations in eastern South Dakota, one in June 2021 and the other in August 2022. Only adults were collected, suggesting they may have been incidentally transported by mammalian or avian hosts. Thorough surveillance only detected *A. maculatum* twice out of a total 2,865 ticks collected across four years, and therefore established populations of this species seem unlikely in South Dakota. Future surveillance should occur in the southern counties where the range of *A. maculatum* may eventually extend northward from Nebraska. While not currently a public health threat, public health officials should be made aware of the potential for non-endemic tick-borne diseases to occur in South Dakota. Transport of humans, livestock, and companion animals can facilitate the spread of *A. maculatum* and pathogens associated with this species (Teel et al. 2010).

Our data suggest only a weak positive correlation between total number of ticks collected and the effort spent obtaining those ticks using the tick drag method. Four years of surveillance data indicates that the optimal duration for conducting tick surveillance is between 1-3 person-hours (Figure 3). Interestingly, when time spent collecting ticks exceeds 4 person-hours, values of total ticks were lower than the projected values based on the regression model. These results could be useful for future studies involving surveillance of host-seeking ticks, to ensure that ample time is spent to collect the ticks but not exceeding the time required to get an idea of what ticks are present. Our study did not set a minimum or maximum time constraint for our surveillance, so the effort in person-hours was variable between sites and instances of surveillance. Based on Figure 3, I would recommend setting a minimum constraint of 1 person-hour and a maximum constraint of 2.5-3 person-hours, and to remain consistent timewise between surveillance instances. An optimal surveillance time in person-hours could be determined with additional tick surveillance data, providing more meaningful and robust results in future studies.

Tick-borne illnesses are an emerging public health threat in the midwestern United States, and this study provides new evidence for expanded ranges of two prominent vector species in eastern South Dakota. These species are now known to be present in multiple counties, which can have implications for public health especially in the regions where these ticks have confirmed established populations. Range expansion of ticks may be due to numerous factors, including climate change, habitat disturbance, and vertebrate host range expansion. Our work demonstrates the importance of annual surveillance, especially in areas that have newly established populations or are susceptible to further invasion.

The tick species that humans regularly encounter are all competent vectors of potentially disabling and deadly pathogens, and our findings may be useful for informing preventative measures for reducing tick-borne illness in South Dakota. The author recommends outreach and other education efforts to better inform the public about tick identification and the potential risks of tick-borne illness associated with the species they may encounter. Signage in areas where medically important species are confirmed to be reported or established can help the public take precautions during outdoor recreation. South Dakota would ultimately benefit from further studies and newer models for predictions of future tick range expansion, host distribution, and pathogen prevalence.

Tables

Table 1: Total numbers of each tick species collected during four surveillance years. Combined tick surveillance effort for each year is represented as person-hours. 2019 and 2020 data are extracted from Black et al. 2021.

Year	**No. of *D. variabilis***	**No. of *I. scapularis***	**No. of *A. americanum***	**No. of *A. maculatum***	**Total Ticks**	**Total Person-Hours**	**Ticks Per Person-Hour**
2019	234	22	15	0	271	32.93	8.23
2020	208	7	46	0	261	37	7.05
2021	1022	51	227	1	1301	143.51	9.06
2022	868	51	112	1	1032	83.757	12.32
Total	2332	131	400	2	2865	297.197	9.64

Figures

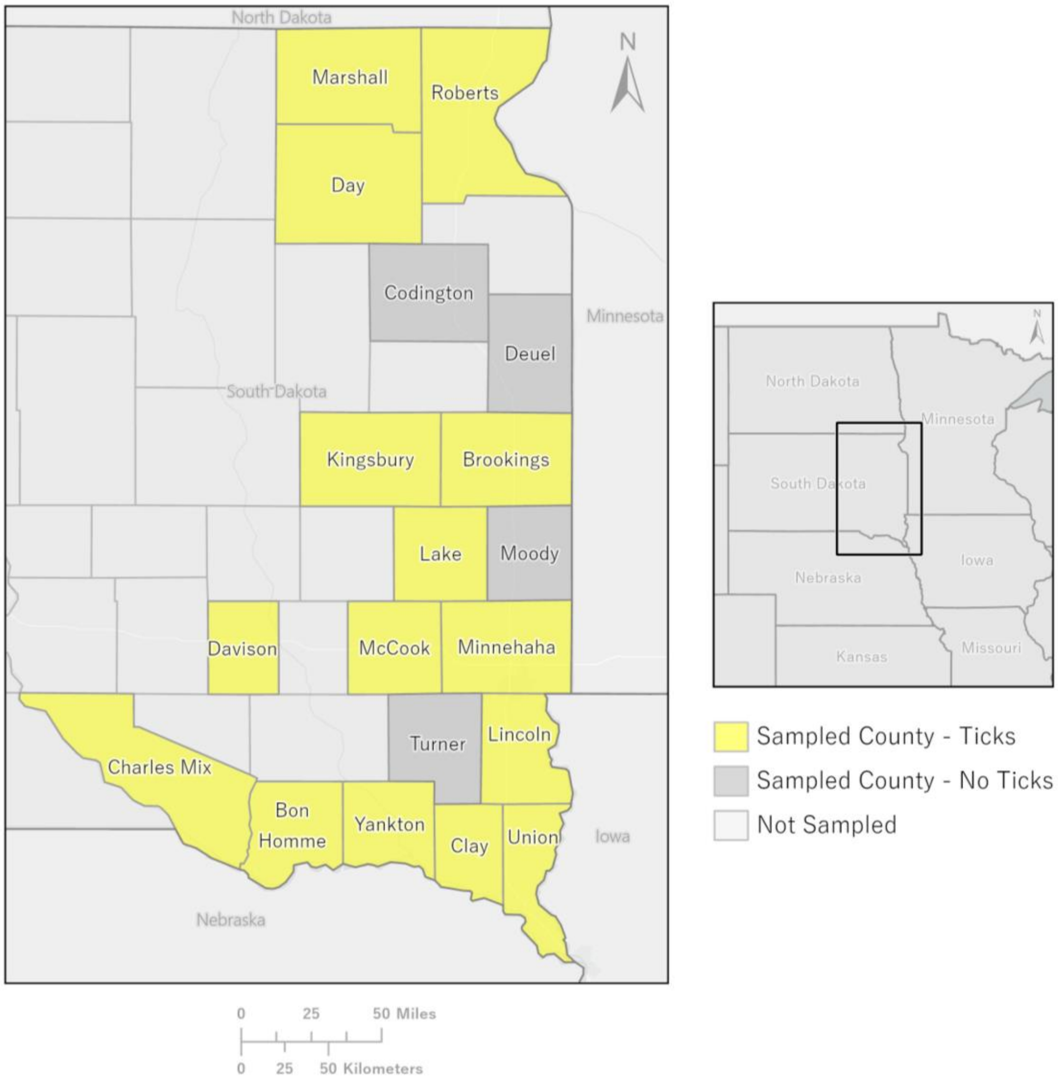

Figure 1: Map of all the counties where surveillance took place within the 2019-2022 sampling seasons. Surveillance was restricted to the East River region and did not occur west of Charles Mix County. This region of the state represents our surveillance boundary, outside of which sampling did not occur.

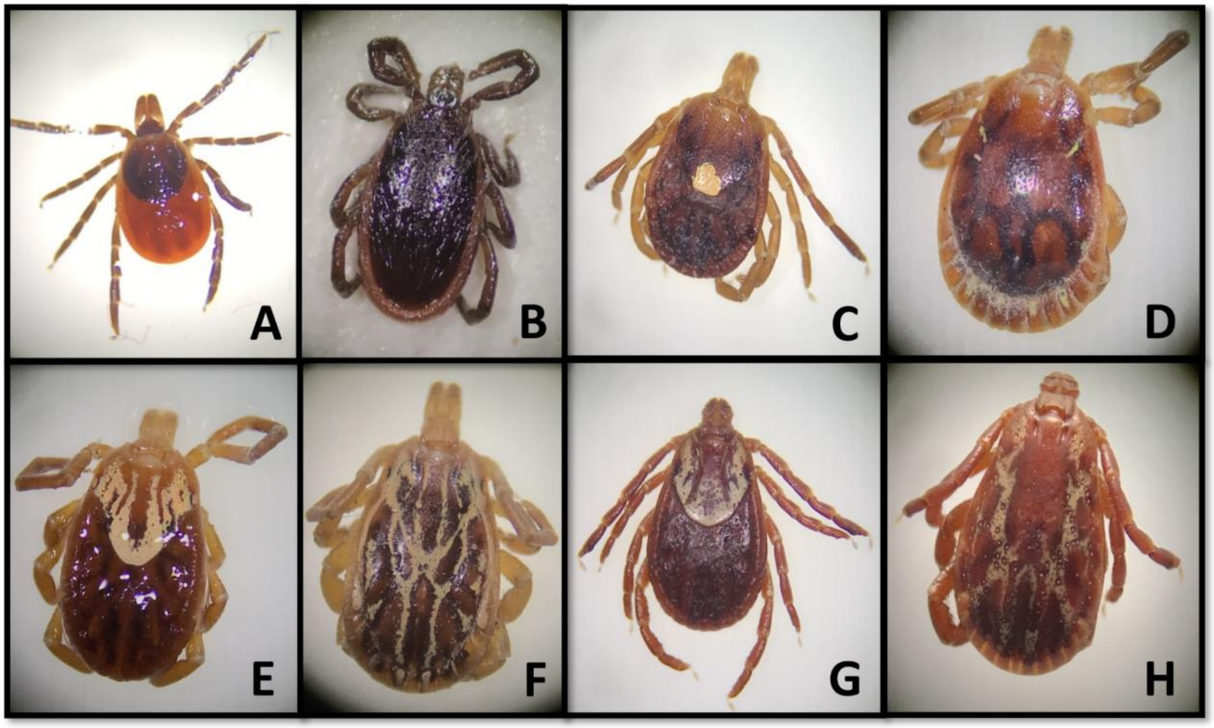

Figure 2: Mature adult specimens of the tick species collected during the 2019-2022 sampling periods: *Ixodes scapularis* (A: Female, B: Male), *Amblyomma americanum* (C: Female, D: Male), *Amblyomma maculatum* (E: Female, F: Male), *Dermacentor variabilis* (G: Female, H: Male). Images are of preserved specimens collected during the 2021-2022 sampling periods.

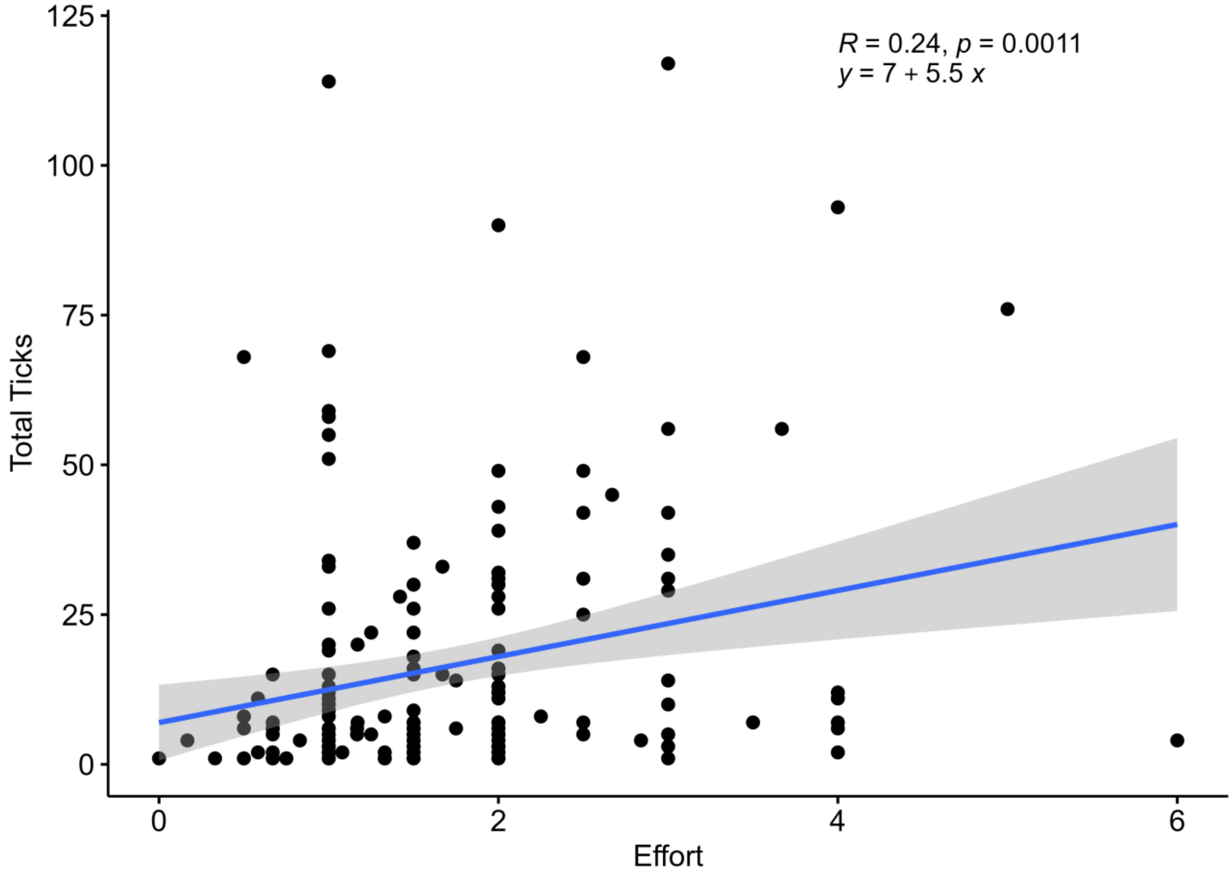

Figure 3: Values of total adult and nymphal ticks collected are plotted against the person-hours spent to collect those ticks (Effort) from 2019-2022. Each data point represents a distinct surveillance instance consisting of a unique location and date, and all ticks collected from that period are summed. Larval tick collections of over 75 individuals were considered “tick bombs” and were not included. R value of 0.24 suggests a weak positive linear relationship between total ticks collected and person-hours taken to collect them.

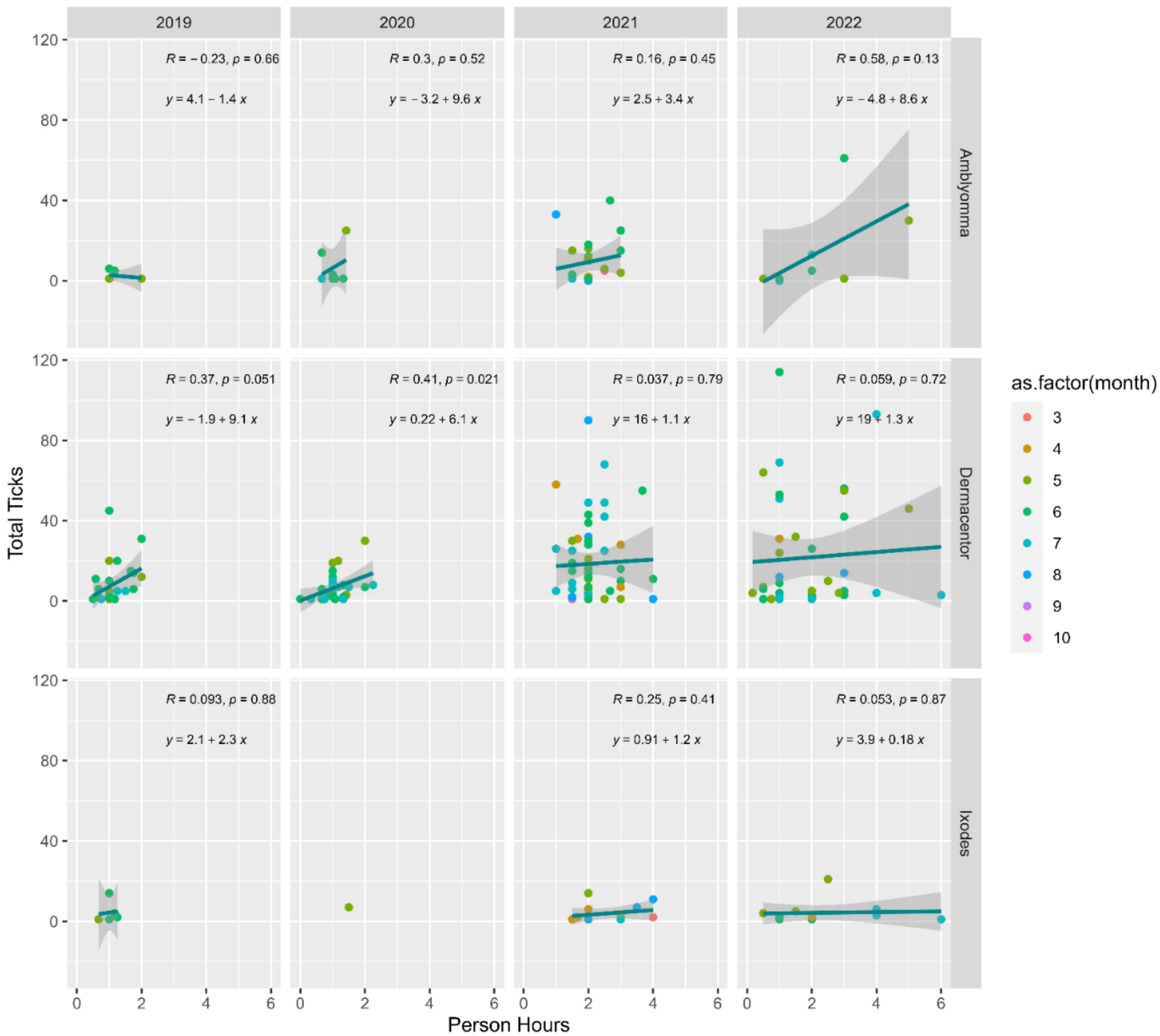

Figure 4: Total number of ticks collected for each species are plotted against person-hours spent to collect them, for each of the surveillance years. Each data point represents a distinct surveillance instance consisting of a unique location and date. Data points are colored to represent the month in which the collection of that data took place.

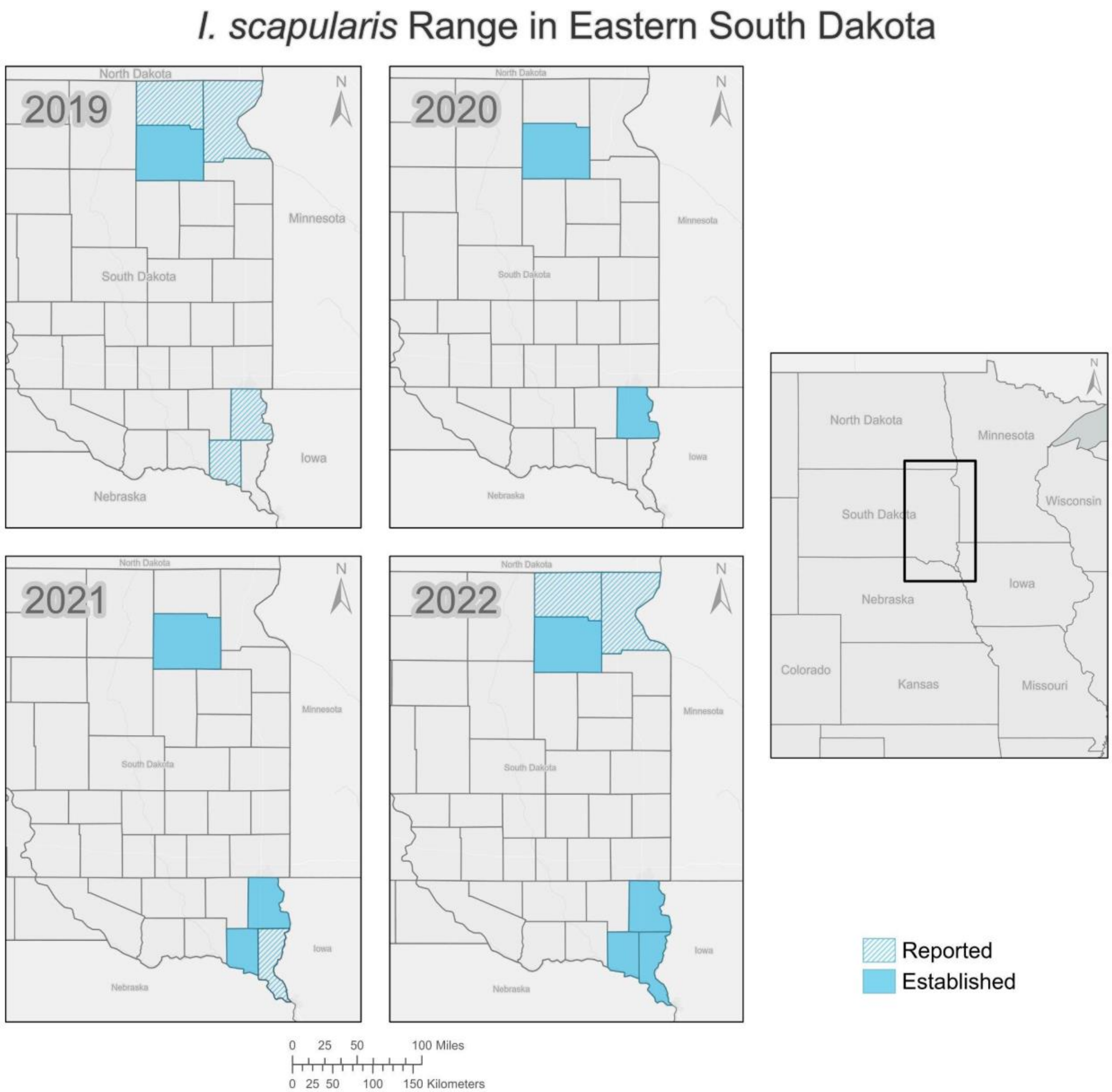

Figure 5: Range map for *Ixodes scapularis* in eastern South Dakota; counties with established populations are filled in blue, and counties where *I. scapularis* was collected but not considered established are filled in with diagonal blue lines. Established counties are retained year-to-year, even if surveillance did not occur in that county in a year after establishment was confirmed. County names can be found in **Figure 1**.

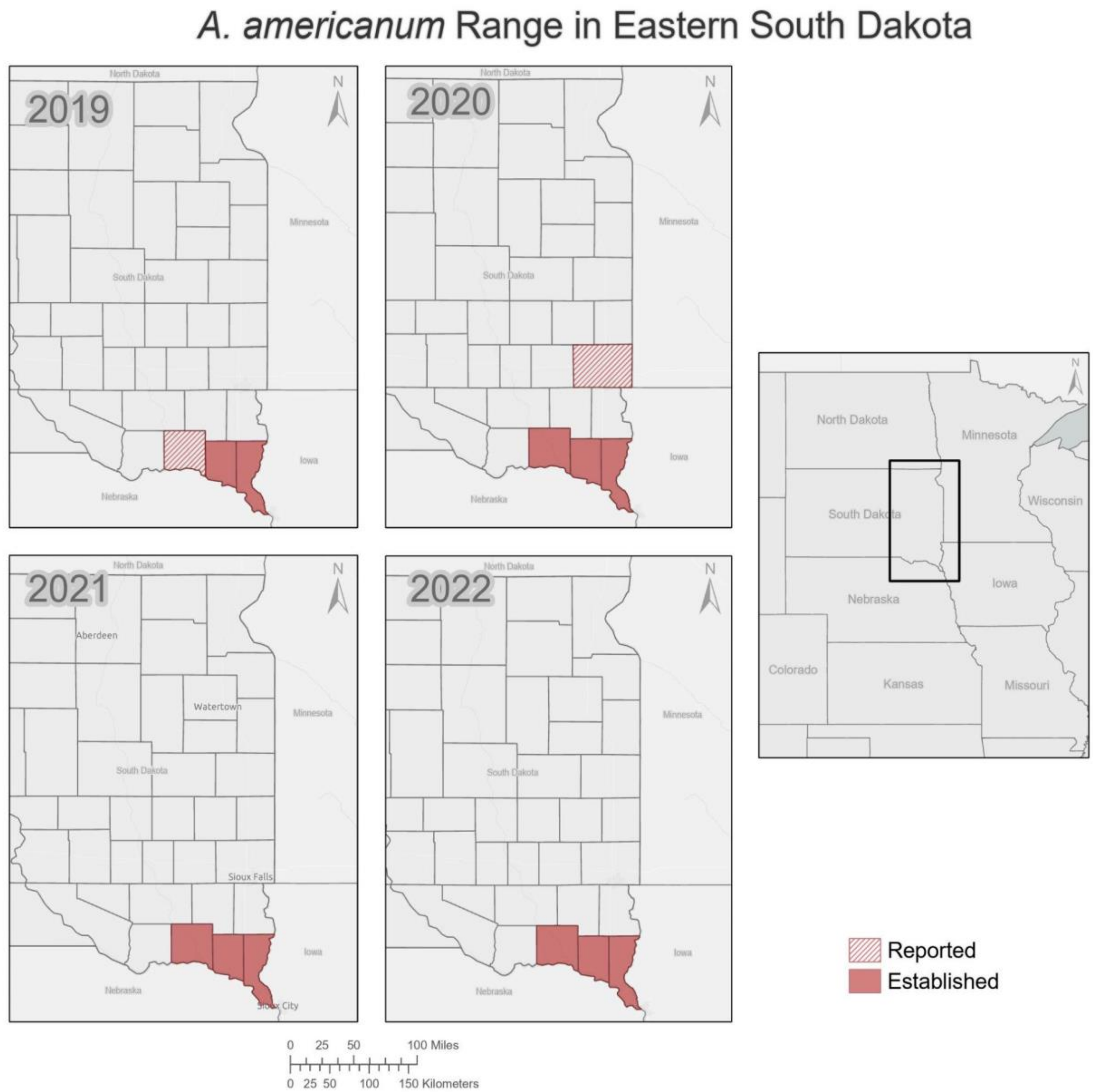

Figure 6: Range map for *Amblyomma americanum* in eastern South Dakota; counties with established populations are filled in red, and counties where *A. americanum* was collected but not considered established are filled in with diagonal red lines. Established counties are retained year-to-year, even if surveillance did not occur in that county in a year after establishment was confirmed. County names can be found in **Figure 1**.

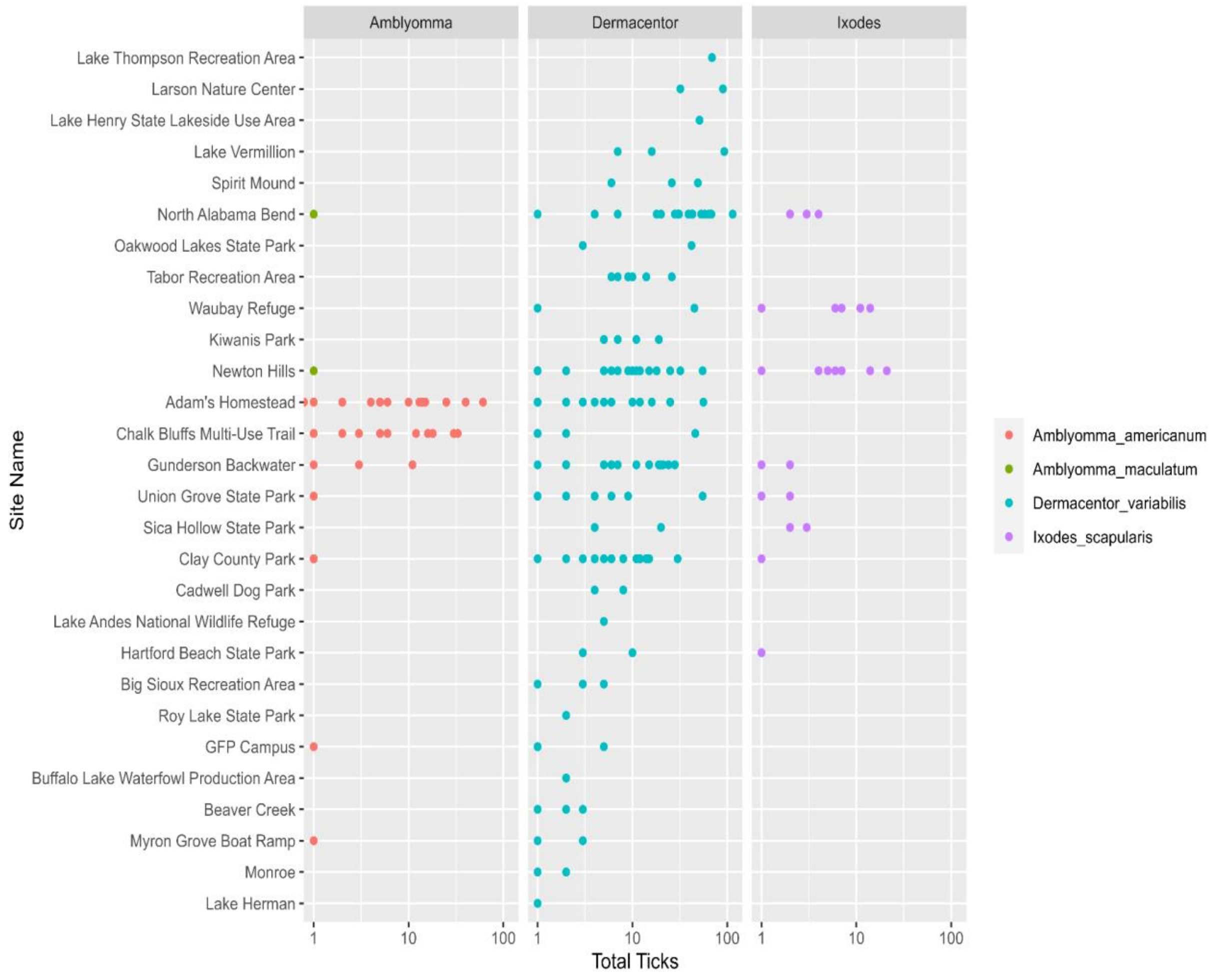

Figure 7: Total adult, nymph, and larval ticks collected at each surveillance site across all years, separated by genus. Each point in a genus column represents a distinct surveillance instance in which at least one tick of that genus was collected. Total ticks are shown on a logarithmic scale*. Surveillance instances where no ticks were collected are not plotted, including sites where ticks were never collected.

*Large numbers of larval *D. variabilis* were collected at Larson Nature Center, and large numbers of *A. americanum* were collected at Adam's Homestead.

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