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## TICK SURVEILLANCE AND PATHOGEN DETECTION IN EASTERN SOUTH DAKOTA

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TICK SURVEILLANCE AND PATHOGEN DETECTION IN EASTERN SOUTH  
DAKOTA

by  
Holly Black

A Thesis Submitted in Partial Fulfillment  
Of the Requirements for the  
University Honors Program

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Department of Biology  
The University of South Dakota  
May 2022

The members of the Honors Thesis Committee appointed

to examine the thesis of Holly Black

find it satisfactory and recommend that it be accepted.

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## ABSTRACT

### Tick Surveillance and Pathogen Detection in Eastern South Dakota

Holly Black

Director: Hugh Britten, Ph.D.

Tick-borne diseases threaten the health of humans, animals, and ecosystems, but surveillance efforts are lacking in some regions. Throughout 2019, 2020, and 2021, we conducted tick surveys aimed at determining the status of the black-legged tick (*Ixodes scapularis*), the American dog tick (*Dermacentor variabilis*) and the lone star tick (*Amblyomma americanum*) in eastern South Dakota. Each year, ticks were collected using a flagging method and identified in the lab. A subset were tested for the presence of *Borrelia burgdorferi*, the causative agent of Lyme disease, by polymerase chain reaction (PCR). *Dermacentor variabilis* was the most commonly identified tick, present in all counties surveyed. *Amblyomma americanum* was also identified in three counties, providing the first evidence of established populations in the state and expanding the range of this species. In addition, established populations of *Ixodes scapularis* were identified in three counties, confirming a previous report of an established population in the state. Adult *I. scapularis* from Lincoln and Clay Counties were found to harbor *B. burgdorferi* during each surveillance year, suggesting the ongoing presence of the pathogen in tick populations in the state and representing its southwestern-most detection in the Midwest United States. These findings provide important information for assessing and monitoring the public health risk from tick-borne diseases in an area with minimal surveillance.

**KEYWORDS:** South Dakota, *Ixodes scapularis*, *Borrelia*, Lyme, infection, expansion, *Amblyomma americanum*

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## INTRODUCTION

Tick-borne diseases are increasingly recognized as an emerging public health threat in the United States. In recent years, several initiatives to support research on ticks and tick-borne diseases have been taken by national public health agencies (Eisen 2020). Nonetheless, surveillance and information remain limited in some regions of the country. The state of South Dakota is one such location, as vector control infrastructure in the state is lacking (NACCHO 2017). Understanding the regional ecology of ticks and tick-borne pathogens is important for assessing and monitoring public health risk and for targeting interventions. Eastern South Dakota and the surrounding localities consist primarily of former tall grass and mixed prairie converted to agriculture, with very little forested land (Walters 2016). This landscape and the continental climate provide suitable habitat for the American dog tick, *Dermacentor variabilis*, and this species of tick is widely distributed throughout the state. *D. variabilis* acts as a vector of *Rickettsia rickettsii* (Rocky Mountain spotted fever) and *Francisella tularensis* (tularemia). Locally acquired cases of these diseases occur sporadically in South Dakota (CDC 2018, Huntington and Allison 2017). Lyme disease, caused by *Borrelia burgdorferi*, and ehrlichiosis, caused by *Ehrlichia spp.*, are seen even less frequently and cases are not thought to be locally acquired (CDC 2018, Huntington and Allison 2017). In the midwestern United States, the black-legged tick, *Ixodes scapularis* is the primary vector of *Borrelia spp.* This tick prefers forested areas and thus much of eastern South Dakota does not provide suitable habitat (Johnson et al. 2016, Guerra et al. 2002). Nonetheless, the distribution of this vector appears to be expanding (Hahn et al. 2016, Alkische et al. 2021) and small patches of land in eastern South Dakota may provide suitable microhabitats to sustain populations

(Alkishe et al. 2021). Isolated reports of *I. scapularis* in the state date back to the 1990s (McDaniel and Hildreth 1992), though these were based on ticks collected from an animal host rather than host-seeking populations. An established field population was documented in 2016 (Maestas et al. 2016) and additional questing ticks have been collected since then (Maestas et al. 2018). However, only a single individual harboring the agent of Lyme disease has been identified in the state (Maestas et al. 2018) and testing is lacking. Similarly, the lone star tick, *Amblyomma americanum*, which vectors multiple species of *Ehrlichia* and also plays a role in alpha-gal meat allergy (Crispell et al. 2019), prefers wooded areas and the edge of its range is thought to lie in the central Midwest, reaching into central Nebraska and northern Iowa (Raghavan et al. 2019). Isolated instances of *A. americanum* collected from an animal host in South Dakota have occurred (Springer et al. 2014, Monzon et al. 2016), but there are no official reports of established host-seeking populations. To address the lack of surveillance and information on ticks in South Dakota, we conducted a survey in the summer of 2019, 2020, and 2021 focusing on the eastern counties. Our goals were to determine if *A. americanum* is undergoing range expansion from the south and to gain additional insight on the presence of *I. scapularis* and *B. burgdorferi*.

## MATERIALS AND METHODS

### **Tick Collection**

Tick collection was conducted at 14 different sites in eight eastern South Dakota counties (Figure 1) from late May to mid-July 2019. Collections took place between 09:00 and 17:00 in temperatures ranging from 15.5° C to 31.1° C and in a variety of conditions, including damp, dry, sunny, and overcast. In 2020, ticks were collected from 15 different

sites in nine counties from mid-May to the end of July. Collections took place between 9:00 and 16:20 in temperatures ranging from 15.6° C to 37.2° C. In 2021, ticks were collected from 16 different sites in nine counties from late March to early October. Collections took place between 7:30 and 15:45 in temperatures ranging from 10.0° C to 31.7° C. Host-seeking ticks were collected by flagging using a flag made of a 1.5 m wooden dowel and a 1-meter square white flannel cloth with a sleeve sewn into it forming a pocket for the dowel (Falco and Fish 1992). The flag was dragged through grasses, understory, and leaf litter at the sample collection sites. Each bout of sampling was timed with approximately 1 hour being spent at each site during a visit. As the ticks clung to the fabric, they were collected with forceps and placed into 1.5 ml microcentrifuge tubes to be transported to the laboratory. In the laboratory, individual ticks were identified under a dissecting microscope using online photo resources (TickEncounter Resource Center) and voucher specimens of common ticks (L. Maestas, State of Delaware). Ticks were identified to species, life stage, and sex. Identification information for each tick was entered into a Microsoft Excel spreadsheet. After identification, the ticks were preserved in 95% ethanol and stored at -4° C.

### **DNA extraction**

DNA was extracted from 21 individual *I. scapularis* ticks collected in 2019, seven collected in 2020, and 24 collected in 2021 to test for the presence of *B. burgdorferi*. First, individual ticks were removed from storage tubes containing ethanol and allowed to dry. Once dry, ticks were longitudinally sectioned in half using a scalpel that was cleaned with 70% ethanol, 10% bleach, and deionized water between uses. One half of each sectioned tick was retained and stored at -80° C. The second half of each tick was

processed for DNA extraction using a commercial kit (Extract-N-Amp, Sigma Aldrich, St. Louis, MO) following a modified version of the manufacturer's protocol. In brief, individual tick sections were placed into extraction buffer, mashed vigorously with a pestle, and then incubated at room temperature for 30 min. The samples were then inactivated at 95° C and neutralized. The DNA concentration of the samples was quantified using a Qubit fluorometer (ThermoFisher, Waltham, MA) to ensure that extraction of sufficient quantities of DNA was achieved. Using this protocol, DNA yields were 3.74-6.3 ng/ µl per tick section, consistent with other extraction protocols (Ammazzalorzo et al. 2015).

### **Polymerase Chain Reaction (PCR)**

DNA extracted from individual *I. scapularis* was tested for the presence of *Borrelia burgdorferi* by polymerase chain reaction (PCR). First, primers targeting a conserved 131 bp region of the *Borrelia spp.* 16S rRNA gene were used under previously described cycling conditions (Wilhelmsson et al. 2010). The manufacturer's protocol for amplification using the Extract-N-Amp kit (Sigma Aldrich) was followed. For each reaction, 4 µl of extracted DNA sample was combined with 1 µl of forward primer, 1 µl of reverse primer, 4 µl of PCR grade water, and 10 µl of amplification mastermix. In addition, a nested PCR assay was independently performed on the same samples using previously described primers that are highly specific for *Borrelia burgdorferi* and amplify a 392 bp region of the ospA gene (Priem et al. 1997). Forty cycles of 94° C for 1 min, followed by 42° C for 1 min and 72° C for 1 min were run with the outer primer set. Twenty-five cycles of the same program were then run using the inner primer set and products from the first reaction as a template for nested reactions. Negative no template

controls (water) and positive controls consisting of DNA from the B31 strain of *B. burgdorferi* were included at each step. Amplicons were visualized by ultraviolet illumination after electrophoresis on agarose gels containing SYBR safe dye. Samples that produced amplicons of expected sizes with both the genus-specific 16S rRNA primers and *B. burgdorferi*-specific ospA primers were considered positive. Finally, ospA amplicons from positive samples were cloned into a pCR4-TOPO vector (ThermoFisher) that was Sanger-sequenced to confirm the identity of *Borrelia burgdorferi* species.

## RESULTS

### 2019

A total of 37.4 person-hours was spent collecting 266 ticks in 2019 resulting in an average of 7.1 ticks per person-hour of surveillance. Details of collections are given in Table 1. Three species were identified. Of the 266 ticks collected during 2019, 229 (86.1%) were *D. variabilis* while 22 (8.3%) were *I. scapularis* and 15 (5.6%) were *A. americanum*. *D. variabilis* was collected at all sites. On the other hand, *I. scapularis* was detected at five sites in five different counties and *A. americanum* was detected at three sites in three different counties. In three of the five sites where *I. scapularis* was detected, multiple sexes or life stages were collected, including 12 adults and two nymphs at one site in Day County (Table 2). Similarly, multiple sexes or life stages of *A. americanum* were collected in two of the three sites where this species was detected, including nine nymphs and three adults at one site in Union County (Table 2). Twenty-one of the *I. scapularis* ticks collected in 2019 were subjected to DNA extraction and PCR to detect the presence of *B. burgdorferi*. In this screen, two of 21 individuals (9.5%) were positive.

One was an adult female collected in Day County and the other was an adult male collected in Lincoln County. BLAST analysis of the ospA gene amplicons obtained from the two positive samples revealed 99.8% and 99.4% sequence identity between these sequences and that of the ospA gene of *B. burgdorferi* subtype 1d2 (GenBank: KM069324.1), which was the closest match found in GenBank.

Table 1. Tick collection data from 2019 by county and site sampled in eastern South Dakota.

\*County totals are in bold.

Location	<i>No. of Dermacentor</i>	<i>No. of Ixodes</i>	<i>No. of Amblyomma</i>	No. of total ticks	Total person hours	Ticks/person hours
<b>Clay County</b>	<b>127</b>	<b>1</b>	<b>2</b>	<b>130</b>	<b>15.09</b>	<b>8.61</b>
<i>Clay County Park</i>	59	0	2	61	6.5	9.38
<i>Gunderson Backwater</i>	12	1	0	13	4.92	2.64
<i>North Alabama Bend</i>	50	0	0	50	3	16.67
<i>Spirit Mound</i>	6	0	0	6	0.67	8.96
<b>Union County</b>	<b>9</b>	<b>0</b>	<b>12</b>	<b>21</b>	<b>7.41</b>	<b>2.83</b>
<i>Union Grove State Park</i>	6	0	0	6	3.25	1.85
<i>Adam's Homestead</i>	3	0	12	15	4.16	3.61
<b>Day County</b>	<b>48</b>	<b>14</b>	<b>0</b>	<b>62</b>	<b>2</b>	<b>31</b>
<i>Waubay National Wildlife Refuge</i>	48	14	0	62	2	31
<b>Roberts County</b>	<b>10</b>	<b>1</b>	<b>0</b>	<b>11</b>	<b>2</b>	<b>5.5</b>
<i>Hartford Beach State Park</i>	10	1	0	11	2	5.5
<b>Marshall County</b>	<b>20</b>	<b>2</b>	<b>0</b>	<b>22</b>	<b>3.67</b>	<b>5.99</b>
<i>Sica Hollow State Park</i>	18	2	0	20	2.5	8
<i>Roy Lake State Park</i>	2	0	0	2	1.17	1.71
<b>Lincoln County</b>	<b>7</b>	<b>4</b>	<b>0</b>	<b>11</b>	<b>4.17</b>	<b>2.64</b>
<i>Newton Hills State Park</i>	7	4	0	11	4.17	2.64
<b>Minnehaha County</b>	<b>6</b>	<b>0</b>	<b>0</b>	<b>6</b>	<b>3.08</b>	<b>1.95</b>
<i>Beaver Creek Nature Area</i>	3	0	0	3	2.08	1.44
<i>Big Sioux Recreation Area</i>	3	0	0	3	1	3
<b>Yankton County</b>	<b>2</b>	<b>0</b>	<b>1</b>	<b>3</b>	<b>1</b>	<b>3</b>
<i>Chalk Bluffs Multi-use Trail</i>	2	0	1	3	1	3
<b>2019 Total</b>	<b>229</b>	<b>22</b>	<b>15</b>	<b>266</b>	<b>37.42</b>	<b>7.11</b>

Table 2. Numbers of nymph, male, and female *Ixodes scapularis* and *Amblyomma americanum* collected in 2019.

\*Based on the criteria for the establishment of *Ixodes scapularis* and *Ixodes pacificus* of the U.S. CDC and (Dennis et al. 1998).

#Population establishment criteria were previously met for *I. scapularis* in Clay County (Maestas et al. 2016).

<i>Ixodes scapularis</i>					
County	Nymph	Male	Female	Total	Established*
Clay	0	1	0	1	Yes <sup>#</sup>
Day	2	9	3	14	Yes*
Lincoln	0	2	2	4	No
Marshall	0	1	1	2	No
Roberts	0	0	1	1	No
<i>Amblyomma americanum</i>					
Clay	1	1	0	2	Yes*
Union	9	1	2	12	Yes*
Yankton	0	0	1	1	No

## 2020

A total of 38.6 person-hours was spent collecting 259 ticks in 2020 resulting in an average of 6.7 ticks per person-hour of surveillance (Table 3). Similar to 2019, three species of tick were identified. Of the 259 ticks collected, 205 (79.2 %) were *D. variabilis* while 7 (2.7%) were *I. scapularis* and 47 (18.1%) were *A. americanum*. *D. variabilis* was found at each of the 15 surveillance sites. *I. scapularis* was only collected at one site in 2020, Newton Hills State Park in Lincoln County. Additionally, *A. americanum* was collected at four sites in four counties including the two counties with established populations in 2019 (Table 4). In both Union and Clay Counties, *A. americanum* of multiple life stages were again collected this year, reconfirming the population characteristics established in 2019. To add to this, one adult female and 3 nymph *A. americanum* were collected from Chalk Bluffs Multi-Use Trail in Yankton County (Table 4). The seven *I. scapularis* collected in 2020 from Newton Hills State Park

were subjected to DNA extraction and PCR to detect the presence of *B. burgdorferi* with the same methodology used to test the 2019 *I. scapularis* samples. Of the 7 samples screened, one (14.3%) adult female was positive.

Table 3. Tick collection data from 2020 by county and site sampled in eastern South Dakota.

\*County totals are in bold.

Location	<i>No. of Dermacentor</i>	<i>No. of Ixodes</i>	<i>No. of Amblyomma</i>	No. of total ticks	Total person hours	Ticks/person hours
<b>Clay County</b>	<b>110</b>	<b>0</b>	<b>2</b>	<b>112</b>	<b>14.5</b>	<b>7.72</b>
<i>Clay County Park Gunderson Backwater</i>	70	0	2	72	10.66	6.75
<i>North Alabama Bend</i>	38	0	0	38	2.84	13.38
<i>Myron Grove</i>	1	0	0	1	.67	1.49
<i>Myron Grove</i>	1	0	0	1	0.33	11.61
<b>Union County</b>	<b>6</b>	<b>0</b>	<b>40</b>	<b>46</b>	<b>4.09</b>	<b>11.24</b>
<i>Union Grove State Park</i>	2	0	0	2	1.00	2.00
<i>Adam's Homestead</i>	4	0	40	44	3.09	14.24
<b>Davison County</b>	<b>49</b>	<b>0</b>	<b>0</b>	<b>49</b>	<b>5.5</b>	<b>8.91</b>
<i>Kiwanis Trail - Mitchell</i>	37	0	0	37	3.50	10.57
<i>Mitchell Dog Park</i>	12	0	0	12	2.00	6.00
<b>Bon Homme County</b>	<b>26</b>	<b>0</b>	<b>0</b>	<b>26</b>	<b>3.33</b>	<b>7.81</b>
<i>Tabor Lake Area</i>	26	0	0	26	3.33	7.81
<b>McCook County</b>	<b>10</b>	<b>0</b>	<b>0</b>	<b>10</b>	<b>4.00</b>	<b>2.50</b>
<i>Lake Vermillion – Montrose, SD</i>	7	0	0	7	2.00	3.50
<i>Monroe, SD</i>	3	0	0	3	2.00	1.50
<b>Lincoln County</b>	<b>1</b>	<b>7</b>	<b>0</b>	<b>8</b>	<b>2.33</b>	<b>3.43</b>
<i>Newton Hills State Park</i>	1	7	0	8	2.33	3.43
<b>Minnehaha County</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>2</b>	<b>1.75</b>	<b>1.14</b>
<i>Sioux Falls Outdoor Campus</i>	1	0	1	2	1.75	1.14
<b>Yankton County</b>	<b>1</b>	<b>0</b>	<b>4</b>	<b>5</b>	<b>2.08</b>	<b>2.40</b>
<i>Chalk Bluffs Multi-use Trail</i>	1	0	4	5	2.08	2.40
<b>Lake County</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1.00</b>	<b>1.00</b>
<i>Lake Herman</i>	1	0	0	1	1.00	1.00
<b>2020 Total</b>	<b>205</b>	<b>7</b>	<b>47</b>	<b>259</b>	<b>38.58</b>	<b>6.71</b>



Table 4. Numbers of nymph, male, and female *Ixodes scapularis* and *Amblyomma americanum* collected in 2020.

\*Based on the criteria for the establishment of *Ixodes scapularis* and *Ixodes pacificus* of the U.S. CDC and (Dennis et al. 1998).

<i>Ixodes scapularis</i>					
County	Nymph	Male	Female	Total	Established*
Lincoln	0	3	4	7	Yes*
<i>Amblyomma americanum</i>					
Clay	1	0	1	2	Yes*
Union	32	5	3	40	Yes*
Yankton	3	0	1	4	Yes*
Minnehaha	0	1	0	1	No

## 2021

Because the 2021 tick collection season spanned from Spring until Fall, unlike the previous years that only surveyed during the Summer, the results will be discussed separately for Spring, Summer, Fall, and then collectively.

### Spring 2021

In the Spring (March 28<sup>th</sup> – May 12<sup>th</sup>) of 2021, a total of 27.2 person-hours was spent collecting 268 ticks. This resulted in an average of 9.9 ticks collected per person-hour of surveillance. Of the 268 ticks collected, 218 (81.3 %) were *D. variabilis* while 25 (9.3%) were *I. scapularis* and 25 (9.3%) were *A. americanum*. *D. variabilis* was detected at 7 of the surveillance sites across 5 counties, *I. scapularis* was detected at 5 sites across 3 counties, and *A. americanum* was detected at 3 sites from 3 different counties during Spring of 2021.

## **Summer 2021**

During the Summer (May 29<sup>th</sup> – August 1<sup>st</sup>) of 2021, a total of 70.1 person-hours was spent collecting 817 ticks resulting in an average of 11.7 ticks collected per person-hour of surveillance. Of the 817 ticks collected, 666 (81.5 %) were *D. variabilis* while 1 (0.1%) were *I. scapularis* and 150 (18.4%) were *A. americanum*. *D. variabilis* was detected at every surveillance site, *I. scapularis* was detected at 1 site in Day County, and *A. americanum* was detected at 3 sites from 3 different counties during Summer of 2021. Additionally, one male Gulf Coast tick, *Amblyomma maculatum*, was collected at Newton Hills State Park in Lincoln County South Dakota during this period.

## **Fall 2021**

For Fall (August 2<sup>nd</sup> – October 3<sup>rd</sup>) of 2021, a total of 20.3 person-hours was spent collecting a total of 277 ticks. This resulted in an average of 13.7 ticks collected per person-hour of surveillance. Of the 277 ticks collected, 130 (46.9 %) were *D. variabilis* while 19 (6.9%) were *I. scapularis* and 128 (46.2%) were *A. americanum*. *D. variabilis* was detected at every surveillance site, *I. scapularis* was detected at 1 site in Day County, and *A. americanum* was detected at 2 sites during Fall of 2021.

## **2021 Collective Results**

In total, 117.5 person-hours was spent collecting a total of 1368 ticks during 2021. This resulted in an average of 11.6 ticks collected per person-hour of surveillance (Table 5). Of the 1362 ticks collected, 1014 (74.4 %) were *D. variabilis* while 45 (3.3%) were *I. scapularis* and 303 (22.3%) were *A. americanum*. *I. scapularis* was detected at six

surveillance sites across four counties in 2021, including Lincoln, Union, Clay, and Day Counties (Table 6). Additionally, *A. americanum* was collected at four sites in three counties, reconfirming the established population status in all three of those counties (Table 6). Twenty-four adult *I. scapularis* collected in 2021 were subjected to DNA extraction and PCR to detect the presence of *B. burgdorferi* with the same methodology used to test the 2019 *I. scapularis* samples. Of the 24 samples screened, three (12.5%) individuals were positive. Two adult females were from Newton Hills State Park in Lincoln County and one adult male was from Gunderson Backwater in Clay County. South Dakota.

Table 5. Tick collection data from 2021 by county and site sampled in eastern South Dakota.

#Denotes detection of *A. maculatum*, the Gulf Coast Tick.

\*County totals are in bold.

Location	<i>No. of Dermacentor</i>	<i>No. of Ixodes</i>	<i>No. of Amblyomma</i>	No. of total ticks	Total person hours	Ticks/person hours
<b>Clay County</b>	<b>620</b>	<b>10</b>	<b>16</b>	<b>646</b>	<b>34.91</b>	<b>18.50</b>
<i>Clay County Park</i>	20	1	2	23	3.75	6.13
<i>Gunderson Backwater</i>	86	2	14	102	7.25	14.07
<i>North Alabama Bend</i>	390	7	0	397	20.66	19.22
<i>Spirit Mound</i>	124	0	0	124	3.25	38.15
<b>Union County</b>	<b>85</b>	<b>1</b>	<b>206</b>	<b>292</b>	<b>28.07</b>	<b>10.40</b>
<i>Union Grove State Park</i>	0	1	0	1	2.00	0.50
<i>Adam's Homestead</i>	85	0	206	291	26.07	11.16
<b>Davison County</b>	<b>5</b>	<b>0</b>	<b>0</b>	<b>5</b>	<b>1.00</b>	<b>5.00</b>
<i>Kiwanis Trail - Mitchell</i>	5	0	0	5	1.00	5.00
<b>Day County</b>	<b>1</b>	<b>20</b>	<b>0</b>	<b>21</b>	<b>12.5</b>	<b>1.68</b>
<i>Waubay National Wildlife Refuge</i>	1	20	0	21	12.5	1.68
<b>McCook County</b>	<b>16</b>	<b>0</b>	<b>0</b>	<b>16</b>	<b>2.00</b>	<b>8.00</b>
<i>Lake Vermillion – Montrose, SD</i>	16	0	0	16	2.00	8.00
<b>Lincoln County</b>	<b>162</b>	<b>14</b>	<b>1<sup>#</sup></b>	<b>177</b>	<b>20.75</b>	<b>8.53</b>
<i>Newton Hills State Park</i>	161	14	1 <sup>#</sup>	176	19.75	8.91
<i>Sioux Falls Outdoor Campus</i>	1	0	0	1	1.00	1.00
<b>Minnehaha County</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>1.00</b>	<b>2.00</b>
<i>Buffalo Lake Waterfowl Production Area</i>	2	0	0	2	1.00	2.00
<b>Yankton County</b>	<b>3</b>	<b>0</b>	<b>81</b>	<b>84</b>	<b>11.25</b>	<b>7.46</b>
<i>Chalk Bluffs Multi-use Trail</i>	3	0	81	84	11.25	7.46
<b>Brookings County</b>	<b>125</b>	<b>0</b>	<b>0</b>	<b>125</b>	<b>6.00</b>	<b>20.83</b>
<i>Oakwood Lakes State Park</i>	3	0	0	3	2.00	1.50
<i>Larson Nature Center</i>	122	0	0	122	4.00	30.50
<b>2021 Total</b>	<b>1019</b>	<b>45</b>	<b>304</b>	<b>1368</b>	<b>117.48</b>	<b>11.64</b>

Table 6. Numbers of larva, nymph, male, and female *Ixodes scapularis*, *Amblyomma maculatum*, and *Amblyomma americanum* collected in 2021.

\*Based on the criteria for the establishment of *Ixodes scapularis* and *Ixodes pacificus* of the U.S. CDC and (Dennis et al. 1998).

<i>Ixodes scapularis</i>						
County	Larva	Nymph	Male	Female	Total	Established*
Lincoln	0	0	7	7	14	Yes*
Clay	0	0	5	5	10	Yes*
Union	0	0	1	0	1	No
Day	15	5	0	0	20	Yes*
<i>Amblyomma americanum</i>						
Clay	0	14	1	1	16	Yes*
Union	92	98	4	12	206	Yes*
Yankton	35	39	4	3	81	Yes*
<i>Amblyomma maculatum</i>						
Lincoln	0	0	1	0	1	No

## DISCUSSION

The results of the tick surveillance efforts present several new findings. Combined with the results of other recent tick surveys (Maestas et al. 2016, Maestas et al. 2018), there is a significant need to improve monitoring for ticks and tick-borne pathogens in eastern South Dakota and the surrounding region. Notably, at two sites in 2019, we collected more than six individuals or multiple life stages of *A. americanum*, meeting criteria accepted by the United States Centers for Disease Control and Prevention (US CDC) for classification of a population as established (Dennis et al. 1998). There is one previous report of *A. americanum* larvae and nymphs collected from an unknown host in South Dakota in 1997 (Springer et al. 2014, Monzon et al. 2016) and some isolated unofficial observations of this species have been made in the state. However, to the best of our knowledge, our collections at sites in Clay and Union Counties provide the first evidence of established questing populations of this important pathogen vector in South

Dakota, suggesting that *A. americanum* is expanding its range northward from adjacent states such as Nebraska and Iowa, as has been predicted by models (Alkishe et al. 2021). Additionally, in 2020, more than six individuals or multiple life stages of *A. americanum* were also collected at a site in Yankton County, providing more evidence for its geographical range expansion. Surveillance efforts in 2020 and 2021 reinforced these observations as the criteria for an established population of *A. americanum* were again met each year.

Prior to our survey, an established population of *I. scapularis* in South Dakota was first reported in Clay County (Maestas et al. 2016). *Ixodes scapularis* was also previously detected in six other counties (Brookings, Codington, Lincoln, Marshall, Roberts, and Union) at numbers that did not meet the criteria for population establishment (Maestas et al. 2018, Maestas 2019). The present report confirms an established population of *I. scapularis* in a new county (Day) in South Dakota for the first time, based on the collection of 12 adults and two nymphs at Waubay National Wildlife Refuge. In addition, a single male *I. scapularis* was collected in Clay County where Maestas et al. determined that an established population was present in previous years (Maestas et al. 2016), supporting this prior observation. In 2020, seven adult *I. scapularis* were collected at Newton Hills State Park in Lincoln County, which, unlike the previous year, meets the criteria for an established population in that county. The subsequent surveillance efforts in 2021 support these findings since established population criteria were met again in Day, Lincoln, and Clay Counties.

It should be noted that in 2020, tick surveillance efforts were limited in scope due to the COVID-19 pandemic. The lack of extended and overnight travel to the northern

portion of eastern South Dakota accounts for the lapse in tick surveillance data from Day County and other northern sites. In 2021, the tick surveillance season was newly extended beyond the summer months, beginning in late March and concluding in early October. This provided insight into the seasonal activity of the different life stages and species of the ticks being studied. In late August of 2021, there were instances where hundreds to over a thousand larval *A. americanum* ticks were collected on the flag and these few instances represent outliers. Accurately accounting for hundreds of larval ticks is difficult so these results were omitted from the ticks/person hour calculations, however smaller groups of larval ticks were included. Since larval ticks were not observed in 2019 and 2020 when surveillance efforts ended August 1<sup>st</sup>, these instances in 2021 provide insight into the peak activity of larval *A. americanum* ticks in Union and Yankton Counties.

Interestingly, in 2019 we identified *I. scapularis* harboring *B. burgdorferi* in both Day County and Lincoln County. Identification of *B. burgdorferi* infected *I. scapularis* from the site in Lincoln County continued in 2020 and 2021 providing evidence for the continual presence of the pathogen there. Additionally, in 2021 an *I. scapularis* was found positive in Clay County. There is only one previous report of *B. burgdorferi* infected *I. scapularis* in the state of South Dakota. This finding consisted of the detection of a single infected female in the northeastern county of Marshall (Maestas et al. 2018). Our new county records of *B. burgdorferi* infection add to the single previous record and these repeat detections suggest an ongoing presence of the pathogen in *I. scapularis* populations in South Dakota. Moreover, based on the most recent U.S. CDC report (Fleshman et al. 2021), our detection in Clay County is the southwestern-most detection

of a *B. burgdorferi*-infected *I. scapularis* in the midwestern United States, even more so than our detection in Lincoln County in 2019, forecasting the potential westwardly expansion of Lyme disease cases in the future (Black et al. 2021).

Together, our results indicate that both *I. scapularis* and *A. americanum* are undergoing range expansions into South Dakota. Although habitats in eastern parts of the state are suboptimal, factors such as climate change (Ostfeld and Brunner 2015) and the influx of animals such as deer and mice from neighboring regions could all contribute to this movement. However, the precise factors at play remain to be determined.

Given that both *I. scapularis* and *A. americanum* are vectors of important and emerging pathogens impacting not only humans but also animals, expanding tick and tick-borne pathogen surveillance efforts in the eastern and southern parts of South Dakota is recommended. These efforts can help properly inform public health efforts as well as medical and veterinary diagnoses. In turn, continued tick surveillance efforts will help protect recreators and their pets from the risk of tick encounters at popular nature and state recreational areas.



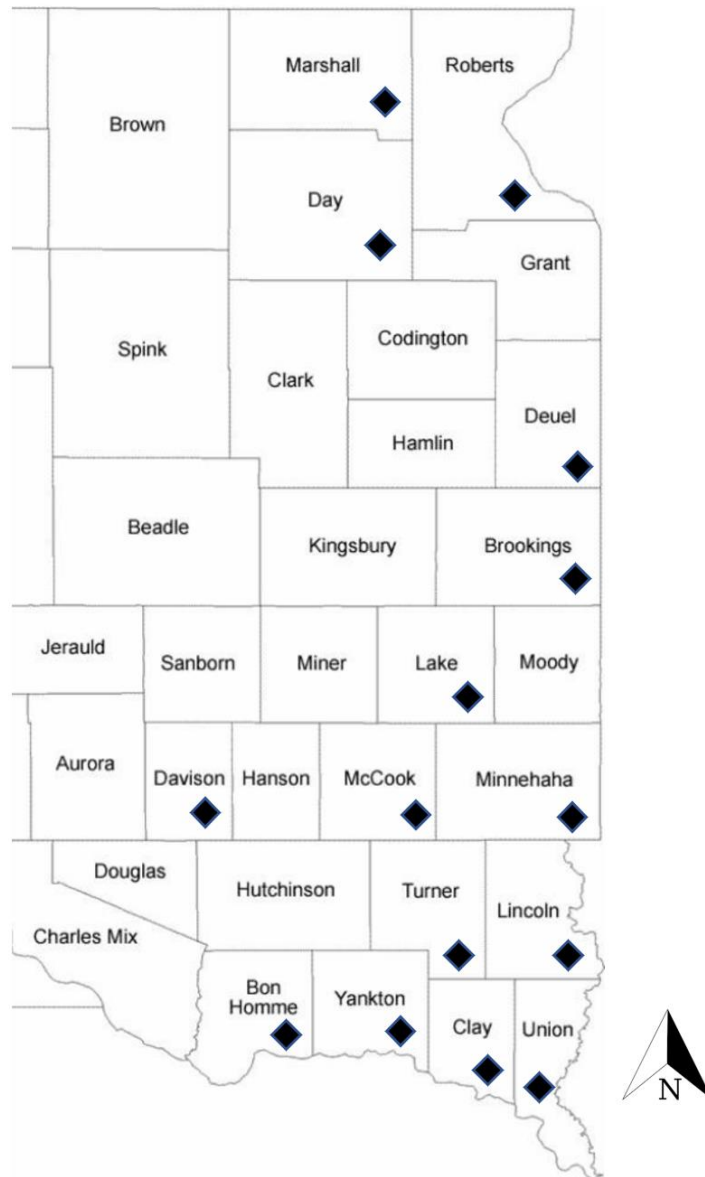


Figure 1. Map of eastern South Dakota with diamonds indicating the counties where tick surveillance took place.

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