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# Detection of *Borrelia miyamotoi* and Powassan Virus Lineage II (Deer Tick Virus) from *Odocoileus virginianus* Harvested *Ixodes scapularis* in Oklahoma

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## Abstract

*Odocoileus virginianus* (white-tailed deer) is the primary host of adult *Ixodes scapularis* (deer tick). Most of the research into *I. scapularis* has been geographically restricted to the northeastern United States, with limited interest in Oklahoma until recently as the *I. scapularis* populations spread due to climate change. Ticks serve as a vector for pathogenic bacteria, protozoans, and viruses that pose a significant human health risk. To date, there has been limited research to determine what potential tick-borne pathogens are present in *I. scapularis* in central Oklahoma. Using a one-step multiplex real-time reverse transcription-PCR, *I. scapularis* collected from white-tailed deer was screened for *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Babesia microti*, and deer tick virus (DTV). Ticks ($n = 394$) were pooled by gender and life stage into 117 samples. Three pooled samples were positive for *B. miyamotoi* and five pooled samples were positive for DTV. This represents a minimum infection rate of 0.8% and 1.2%, respectively. *A. phagocytophilum*, *B. burgdorferi*, and *B. microti* were not detected in any samples. This is the first report of *B. miyamotoi* and DTV detection in Oklahoma *I. scapularis* ticks. This demonstrates that *I. scapularis* pathogens are present in Oklahoma and that further surveillance of *I. scapularis* is warranted.

**Keywords:** *Ixodes scapularis*, *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Babesia microti*, deer tick virus, *Anaplasma phagocytophilum*

## Introduction

*Odocoileus virginianus* (white-tailed deer) have a large geographic distribution, extending from the 60th parallel of Canada to Panama (Taylor 1961). The primary host of adult *Ixodes scapularis* is *O. virginianus*. *I. scapularis* is also known to be the primary vector of *Borrelia burgdorferi*, the agent that causes Lyme disease (Eisen et al. 2016). Other pathogens known to be vectored by *I. scapularis* include *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Babesia microti*, and deer tick virus (DTV) (Sanchez-Vicente et al. 2019).

Each of these pathogens has been documented in questing ticks and documented to cause human illness, including cases in Oklahoma of Lyme disease (Brackney et al. 2008, Hinten at al. 2008, Reichard et al. 2009, Duell et al. 2013, Dibernardo et al. 2014, Mays et al. 2014, Bakken and Dumler 2015, Lantos 2015, Wagemakers et al. 2015, Hermance and Thangamani 2017, Cross et al. 2018, Krause 2020).

It has been estimated that 476,000 Americans are diagnosed and treated for Lyme disease each year (Kugeler et al. 2021). Lyme disease has now been reported in 49.2% of American counties, which is a 44.7% increase since 1998 when records started (Dubie at al. 2018). As of 2015, 46.8% of Oklahoma counties have reported to have *I. scapularis* populations, this also includes reported cases of Lyme disease in Oklahoma County (Reiner et al. 1991, Dubie et al. 2018). *B. miyamotoi* is an emerging pathogen transmitted by *I. scapularis* ticks that was first isolated from ticks in 1995 (Fukunaga et al. 1995).

All tick species that have the potential to transmit *B. burgdorferi* also have the potential to transmit *B. miyamotoi* (Wormser et al. 2019). Research so far indicates that the prevalence of *B. miyamotoi*-infected ticks is much lower than

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that of *B. burgdorferi* (Barbour et al. 2009). The current geographic distribution of *B. miyamotoi* is potentially much larger than *B. burgdoferi* due to the spirochete's ability to transmit transovarially within ticks (Han et al. 2019). Powassan virus lineage II (POWV) or DTV is a tick-borne encephalitic virus from the family Flaviviridae. In 2010, only 8 cases of DTV disease were reported in the United States, but by 2019, that number has increased to 37 cases a year (CDC 2019). DTV is spread by *I. scapularis* ticks demonstrating the need for surveillance in areas with *I. scapularis* populations.

With the changes in climate, the expansion of human activities, and the already large and expanding distribution of white-tailed deer, human exposure to *I. scapularis* is on the rise (Eisen et al. 2016). The behavior of *I. scapularis* in Oklahoma also makes collecting questing ticks difficult by trapping due to small windows of active time (Dubie at al. 2018). Studies of Oklahoma *I. scapularis* have shown that collecting ticks in the evenings or early mornings directly from hosts provides the best opportunity to collect *I. scpaularis* (Dubie et al. 2018).

Owing to the expansion of the *I. scapularis* geographic range and the known establishment of *I. scapularis* in Oklahoma County, a better understanding of pathogen prevalence within feeding ticks in central Oklahoma is needed (Small et al. 2019). Information on the pathogen prevalence carried by *I. scapularis* in central Oklahoma is lacking, creating a knowledge gap with important public health implications.

## Methods

### *Collection*

With permission from the Arcadia Lake Wildlife management, ticks were collected from 25 deer during bow hunting season, October 1 through December 2018. This collection was done during times of peak adult tick activity (Dubie et al. 2018). Samples were collected by grasping the ticks as close to the surface of the skin as possible and pulling upward with steady and even pressure to keep mouthparts intact for identification. The head, ears, chin, chest, and axillae of the deer were examined for ticks.

The fur was carefully pulled back to visually examine each deer and take an estimate of the total tick load of the deer. Ticks were placed in a vial containing 70% ethanol. Vials were kept in a cooler on ice. The date, gender, weight, and the approximate age of deer, zone harvested, and time of day harvested were recorded (Fig. 1). Ticks were transported to the laboratory at University of Central Oklahoma for identification and stored at −80°C until nucleic acid extraction.

### *Identification*

Tick species were identified as *I. scapularis* based on mouthparts, body markings, and other features as laid out in previous research (Keirans and Litwak 1989, Keirans and Durden 1998). Life stage and gender were also determined. Other tick species collected were stored at −80°C but were not tested.

### *Tick homogenization and DNA/RNA extraction*

*I. scapularis* ticks were pooled by gender and life stage based on the deer they were sampled from. Furthermore, engorged females were pooled separately from nonengorged females. No more than five ticks of any life stage were pooled together. Flat and engorged nymphs were pooled together. The ticks were placed in a screw cap lysis tube with sterile bashing beads and DNA/RNA shield from ZYMO Research to ensure stability of genetic material and homogenized on a bead beater (ZYMO Research 2018). DNA and RNA were extracted and purified using the Quick-DNA/RNA Pathogen Miniprep protocol from ZYMO Research (ZYMO Research 2018).


**FIG. 1.** Map of Lake Arcadia in Edmond, Oklahoma. Stars indicate zones of positive deer.

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*Multiplex real-time reverse transcription-PCR*

Each pathogen target was selected by a unique gene target as previously described including primers specific to POWV (Tokarz et al. 2017). Primers for each gene target and probes are given in Table 1. A one-step real-time reverse transcription-PCR (RT-PCR) as previously described and performed was used to detect pathogen targets (Tokarz et al. 2017). Following the Invitrogen RNA UltraSense protocol, 2.5 $\mu$L of the enzyme mix, 10 $\mu$L of the 5 × reaction mix, 1 $\mu$L of both forward and reverse primers for all 5 targets, 1 $\mu$L of each target probe, 10 $\mu$L of the sample, and 12.5 $\mu$L of DPEC-treated water were used for a total reaction volume of 50 $\mu$L.

The reverse transcription step was performed at 55°C for 15 min followed by incubation at 95°C for 10 min. The PCR consisted of 40 cycles (95°C for 15 s and 60°C for 30 s). The reverse transcriptase-quantitative PCR (RT-qPCR) was performed on a Bio-Rad Bio cfx96 real-time system C1000 touch thermal cycler. Positive and negative controls for each target were included in each RT-qPCR run. Samples positive by RT-qPCR were amplified by traditional PCR using a Bio-Rad C1000 Thermal Cycler and visualized through gel electrophoresis. The identity of positive samples was confirmed using Sanger sequencing through ETON-Biosciences. In each positive pool, only one tick will be assumed to have been infected to determine the minimum infection rate (MIR).

## Results

Four hundred fifty-three total ticks were collected: 394 *I. scapularis*, 33 *Amblyomma americanum* (Lone-star tick), 13 *Ambylomma maculatum* (gulf coast tick), and 13 *Rhipicephalus sanguineus* (brown dog tick). Deer ticks ($n = 394$) were pooled into 117 total samples. Three pools were positive for *B. miyamotoi* and five were positive for DTV by RT-qPCR. This represents a MIR of 0.5% and 0.85%, respectively. MIR was calculated with the assumption that a positive pool contained only one infected tick [number of positive pools/(number of pools tested × size of pool)].

*A. phagocytophilum*, *B. burgdorferi*, and *B. microti* were not detected in any samples. Three tick pools from three individual deer were positive for *B. miyamotoi* and all five positive DTV samples were collected from the same deer. *B. miyamotoi* positive sample pools consisted of males and nymphs. The DTV-positive sample pools consisted of engorged females, nonengorged females, males, and nymphs (Tables 2 and 3).

Sanger sequencing of the positive samples resulted in amplicons that are listed in Table 3. Amplicons of the positive samples were compared using NCBI BLAST to the 3′-UTR of POWV/DTV (acc. no. AY004081.1), and the flagellin B gene from *B. miyamotoi* (acc. no. MK660526.1) (Ebel et al. 2001, Takhampunya et al. 2019). Each BLAST result for DTV had 99% identity and *B. miyamotoi* had 98% identity (Table 3).

## Discussion

Tick-borne diseases such as Lyme disease continue to spread westward from its northeast epicenter due to climate change and are predicted to increase establishment in new areas (Gardner et al. 2020). Migratory birds also have the potential to spread tick-borne diseases and *I. scapularis* ticks

TABLE 1. PATHOGENS AND TARGET GENES WITH PRIMER AND PROBE SEQUENCES

| *Pathogen* | *Disease* | *Gene target* | *Forward and reverse primers* | *Probe* |
|---|---|---|---|---|
| *Borrelia burgdoferi* | Lyme disease | ospA | For-CCTTCAAGTACTCCAGATCCATTG<br>Rev-AACAAAGACGGCAAGTACGATC | 6-FAM-CAACAGTAGACAAGCTTGA-IBFQ |
| *Babesia microti* | Human babesiosis | Cox1 | For-CATCATGCCAGGCCTGTTTG<br>Rev-GAAGAAACCACACAAGAGCAAATGC | YAK-TACTACCCATACTGGTCGGTGCTCC-IBFQ |
| *Anaplasma phagocytophilum* | Human granulocytic anaplasmosis | 16s rRNA | For-GGCATGTAGGCGGTTCGGT<br>Rev-CACTAGGAATTCCGCTATCCTCTCC | TAMRA-GCCAGGGCTTAACCCTGGAGCT-IBRQ |
| *Borrelia miyamotoi* | Tick-borne relapsing fever | Flab | For-AGCACAAGCTTCATGGACATTGA<br>Rev-GAGCTGCTTGAGCACCTTCTC | TxR-TGTGGGTGCAAATCAGGATGAAGCA-IBRQ |
| DTV | Powassan encephalitis | 3′-UTR | For-GTGATGTGGCAGCGCACC<br>Rev-CTGCGTCGGGAGCGACCA | Cy5-CCTACTGCGGCAGCACACACAGTG-IBRQ |

DTV, deer tick virus.

Table 2. The Prevalence of Deer Tick Virus, *Borrelia miyamotoi*, *Borrelia burgdorferi*, *Babesia microti*, and *Anaplasma phagocytophilum* in *Ixodes scapularis* Collected from *Odocoileus virginianus*

| *Tested pools of* Ixodes scapularis | | *Total by tick stage* | | | |
|---|---|---|---|---|---|
| *Pathogens* | *Pools positive* | *EF* | *F* | *M* | *N* |
| DTV | 5/117[a] | 2/96[b] | 1/59 | 1/175 | 1/64 |
| *Borrelia miyamotoi* | 3/117 | 0/96 | 0/59 | 2/175 | 1/64 |
| *Borrelia burgdorferi* | 0/117 | 0/96 | 0/59 | 0/175 | 0/64 |
| *Babesia microti* | 0/117 | 0/96 | 0/59 | 0/175 | 0/64 |
| *Anaplasma phagocytophilum* | 0/117 | 0/96 | 0/59 | 0/175 | 0/64 |

[a]Values correspond to number of PCR-positive tick pools/total number of tick pools tested.
[b]Values correspond to total number of positive ticks by life stage/total number of ticks collected in that life stage (assuming only one tick per pool was positive).
EF, engorged female; F, female; M, male; N, nymphal ticks.

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(Cumbie et al. 2021). Even though the spatial scale and number of ticks collected in this study were relatively small, the lack of *B. burgdorferi* detection is in line with recent research in Oklahoma (Kocan et al. 1992, Dubie et al. 2018). *B. miyamotoi* is commonly found with *B. burgorferi* and can be maintained in the same mammalian hosts (Barbour et al. 2009).

Research into what potential pathogens *Peromyscus leucopus* (white-footed mouse) is carrying in central Oklahoma may help shed light on this. Other potential animals in Oklahoma that have been known to be reservoirs of *B. burgdorferi* include *Turdus migratorius* (American Robins), *Tamias striatus* (chipmunks), *Blarina brevicauda* (short-tailed shrews), and *Sciurus carolinensis* (eastern gray squirrel) (Richter et al. 2000, Salkeld et al. 2008). *I. scapularis* ticks collected from *O. virginianus* have demonstrated that *B. miyamotoi* can persist in the tick population through vertical transmission (Han et al. 2019).

Multiple studies have demonstrated the presence of *B. burgdorferi* in Texas at low prevalence rates (Rawlings and Teltow 1994, Feria-Arroyo et al. 2014, Mitchell et al. 2016). In Missouri, *B. burgdorferi* was successfully isolated and cultivated from *Ixodes* ticks found on *Sylvilagus* spp. (cottontail rabbits) (Oliver Jr et al. 1998). These two neighboring states identifying *B. burgdorferi* in *I. scapularis* lend support to the presence of the closely related *B. miyamotoi* in *I. scapularis* ticks in Oklahoma.

The incidence of *B. microti*-induced Babesiosis has increased in the United States over the past decade (Bloch at al. 2019). Coinfection with *B. microti* and *B. burgdorferi* is also common, demonstrating the ability of these two pathogens to persist in the same tick (Parveen and Bhanot 2019). Models have shown that the presence of *B. burgdorferi* can actually aid in the establishment of *B. microti* (Dunn et al. 2014). *Babesia* spp. has been identified in Oklahoma *Ursus americanus* (black bears) in which *I. scapularis* was also present on the bear (Skinner et al. 2017). The inability to detect *B. microti* from *I. scapularis* in this study potentially demonstrates that different animal reservoirs may be perpetuating *B. microti* in the endemic areas for human Babesiosis, but overall, there is a lack of testing in Oklahoma for *B. microti*.

The presence of DTV in central Oklahoma represents a jump in terms of the previously known geographical range of states with known *I. scapularis* positive results. The large geographic range of white-tailed deer (*O. virginianus*) and the spread of *I. scapularis* ticks in the United States are thought to potentially explain the increase in DTV cases (Campagnolo et al. 2018). North Carolina and North Dakota have had positive cases of DTV encephalitis since 2000 (Campbell and Krause 2020). In New Mexico, *Peromyscus* spp. were seropositive for DTV antibodies (Deardorff et al. 2013).

Powassan virus was isolated from *Dermacentor andersoni* tick in Colorado in 1952 that was later characterized as DTV (Thomas et al. 1960, Dupuis II et al. 2013). DTV-infected white-tailed deer have been documented before in previous studies, which suggests a potential role for these animals in spread of this disease (Campagnolo et al. 2018). No cases of DTV encephalitis have been reported in Oklahoma, so the presence of this flavivirus demonstrates the need to study potential differences in the reservoir hosts in endemic versus nonendemic areas and to conduct further surveillance of the *I. scapularis* population.

Previous studies into *I. scapularis* in Oklahoma have demonstrated an absence of *A. phagocytophilum* (Dubie et al. 2018). The prevalence of *A. phagocytophilum* is increasing and with it human granulocytic anaplasmosis in the United States (Ismail and McBride 2017). Studies have demonstrated that white-tailed deer are suitable natural reservoirs for *A. phagocytophilum* (Dugan et al. 2006). As *I. scapularis* populations continue to spread, proactive surveillance of these pathogens, including *A. phagocytophilum*, is still recommended.

Recent study has suggested that white-tailed deer may play an important role as reservoirs of *B. miyamotoi* and as sentinel indicators of DTV prevalence in a geographic region (Han et al. 2016, Mlera and Bloom 2018). Ticks harvested from white-tailed deer have a significantly higher prevalence rate of *B. miyamotoi* compared with field-harvested ticks (Han et al. 2016). Medium sized mammals are potentially better reservoirs of DTV than the traditional reservoir for *I. scapularis*-associated pathogens of the white-footed mouse (Mlera and Bloom 2018). The inability to isolate deer tick viral particles from the white-footed mouse demonstrates this (Mlera and Bloom 2018).

A medium sized mammal, the woodchuck (*Marmota monax*), has had DTV isolated from blood samples (Mlera and Bloom 2018). White-tailed deer as close as Louisiana have tested positive for DTV antibodies, however, no studies to date have attempted to isolate viral particles from deer (Pedersen et al. 2017, Mlera and Bloom 2018). This research combined with the presence of DTV and *B. miyamotoi* from white-tailed deer-harvested *I. scapularis* in Oklahoma may highlight the potential role this mammal has as a reservoir for *B. miyamotoi* and as a sentinel indicator of DTV.

Table 3. Sequencing Results for Positive Reverse Transcriptase-Quantitative PCR Samples

| *Deer no./life stage (no. of ticks in sample pool)* | *Pathogen* | *Sequence (sequence length in base pairs)* | *GenBank acc. no.* | *% Identity* |
|---|---|---|---|---|
| Deer 6/males (5) | *Borrelia miyamotoi* | **GGCTGC**TGGGTGCATCAGGATGA GCATTGCTGTCAATATTTATTCAGC TAATGTTGCAAATCTTTTTAATGGA GAAGGTGCTCAAGCAGCTCCAGCT CAAGAAGGAGCACAACAGGAGGG AGTTCAAGCAGCTCCTGCTCCAGCA GCCGCTCCAGTTCAAGGTGGAGTT AATTCTCCAATTAATGTTACAACTGC TATTGATGCTAATATGTCACTTTCAA AGATCGAAAAACTGTAATATGTCAC AATGTATTTTTATGCTATAATTCGAG CTCTGCTTATATGTTCCTCCCGTTATC GATGCGGGGTCGAACCAGTTT (223) | MK660526.1 | 98 |
| Deer 15/nymphs (2) | *B. miyamotoi* | **GCTGATG**TGGGTGCATCAGGATGAG CATTGCTGTCAATATTTATTCAGCTAA TGTTGCAAATCTTTTTAATGGAGAAG GTGCTCAAGCAGCTCCAGCTCAAGAA GGAGCACAACAGGAGGGAGTTCAAG CAGCTCCTGCTCCAGCAGCCGCTCCAG TTCAAGGTGGAGTTAATTCTCCAATTA ATGTTACAACTGCTATTGATGCTAATA TGTCACTTTCAAAGATCGAAA (226) | MK660526.1 | 98 |
| Deer 29/males (4) | *B. miyamotoi* | **GCGCC**TGT**G**GGTGCATCAGGATGAG CATTGCTGTCAATATTTATTCAGCTAA TGTTGCAAATCTTTTTAATGGAGAAGG TGCTCAAGCAGCTCCAGCTCAAGAAGG AGCACAACAGGAGGGAGTTCAAGCAG CTCCTGCTCCAGCAGCCGCTCCAGTTCA AGGTGGAGTTAATTCTCCAATTAATGTT ACAACTGCTATTGATGCTAATATGTCAC TTTCAAAGATCGAAA (225) | MK660526.1 | 98 |
| Deer 29/engorged females (5) | DTV | **CGAACGTCGGAC**GGCAGCACACTTAG TGACGGGAAGTGGTCGCTCCCGACGC AACTGGGTCAAAAGGAACTTTGTGAG ACCAAAAGGCCTCCTGGAAGGCTCAC CAGGAGTTAGGCCGTTTAGGAGCCCC CGGGCATAACTCGGGAGGAGGGAGG AAGAAGATTGGCAATCTTCCTCGGGAT TTTTCCGCCTCCTATACTAAATTTCCCC CAGGAAGCTGGGGGGGCGGTTCTTGT TCTCCCTGAGCCACCACCATCCAGGCA CAGACAGCCTGACAAA (267) | AY004081.1 | 99 |
| Deer 29/engorged females (2) | DTV | **AATACGTCAAACC**GGCAGCACACTTAG TGACGGGAAGTGGTCGCTCCCGACGCA ACTGGGTCAAAAGGAACTTTGTGAGACC AAAAGGCCTCCTGGAAGGCTCACCAGGA GTTAGGCCGTTTAGGAGCCCCCGGGCAT AACTCGGGAGGAGGGAGGAAGAAGATT GGCAATCTTCCTCGGGATTTTTCCGCCT CCTATACTAAATTTCCCCCAGGAAGCTG GGGGGGCGGTTCTTGTTCTCCCTG AGCCACCACCATCCAGGCACAGACAGC CTGACAAA (267) | AY004081.1 | 99 |

(*continued*)

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TABLE 3. (CONTINUED)

| *Deer no./life stage (no. of ticks in sample pool)* | *Pathogen* | *Sequence (sequence length in base pairs)* | *GenBank acc. no.* | *% Identity* |
|---|---|---|---|---|
| Deer 29/female (1) | DTV | **AACCTTCCTTCGAGC**GGCAGC**AA**CTTA GTGACGGGAAGTGGTCGCTCCCGACG CAACTGGGTCAAAAGGAACTTTGTGAG ACCAAAAGGCCTCCTGGAAGGCTCACCA GGAGTTAGGCCGTTTAGGAGCCCCCGG GCATAACTCGGGAGGAGGGAGGAAGA AGATTGGCAATCTTCCTCGGGATTTTTC CGCCTCCTATACTAAATTTCCCCCAGGA AGCTGGGGGGGCGGTTCTTGTTCTCCCT GAGCCACCACCATCCAGGCACAGACAGC CTGACAAA (269) | AY004081.1 | 99 |
| Deer 29/males (4) | DTV | **AAAAAACTTTATACG**CAGC**AA**CTT AGTGACGGGAAGTGGTCGCTCCCG ACGCAACTGGGTCAAAAGGAACTT TGTGAGACCAAAAGGCCTCCTGGA AGGCTCACCAGGAGTTAGGCCGTTT AGGAGCCCCCGGGCATAACTCGGGA GGAGGGAGGAAGAAGATTGGCAATC TTCCTCGGGATTTTTCCGCCTCCTAT ACTAAATTTCCCCCAGGAAGCTGGG GGGGCGGTTCTTGTTCTCCCTGAGCC ACCACCATCCAGGCACAGACAGCCT GACAAA (264) | AY004081.1 | 99 |
| Deer 29/nymphs (4) | DTV | **AACCCGTTCTTATCG**CA**T**CACACTT AGTGACGGGAAAGTGGTCGCTCCCG ACGCAACTGGGTCAAAAGGAACTTT GTGAGACCAAAAGGCCTCCTGGAAG GCTCACCAGGAGTTAGGCCGTTTAGG AGCCCCCGGGCATAACTCGGGAGGAG GGAGGAAGAAGATTGGCAATCTTCC TCGGGATTTTTCCGCCTCCTATACTAA ATTTCCCCCAGGAAGCTGGGGGGGCG GTTCTTGTTCTCCCTGAGCCACCACC ATCCAGGCACAGACAGCCTGACAAA (265) | AY004081.1 | 99 |

Bold letters indicate differences among the sequences.

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## Conclusion

As *I. scapularis* continues to spread south and west from the northeastern United States, so too should the research into their associated pathogens. This study demonstrates the presence of *I. scapularis*-associated pathogens, *B. miyamoti* and DTV, that were not previously reported in Oklahoma. Active surveillance of *I. scapularis*-associated pathogens is recommended as the *I. scapularis* population continues to grow and expand with climate change, potentially increasing their prevalence rates in the state and increasing the potential for human exposure to these pathogens.

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## Author Disclosure Statement

No competing financial interests exist.

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