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Research

Vector-Borne Diseases, Surveillance, Prevention

Emerging babesiosis in the mid-Atlantic: autochthonous human babesiosis cases and *Babesia microti* (Piroplasmida: Babesiidae) in *Ixodes scapularis* (Acari: Ixodidae) and *Ixodes keiransi* (Acari: Ixodidae) ticks from Delaware, Maryland, Virginia, West Virginia, and the District of Columbia, 2009 to 2024

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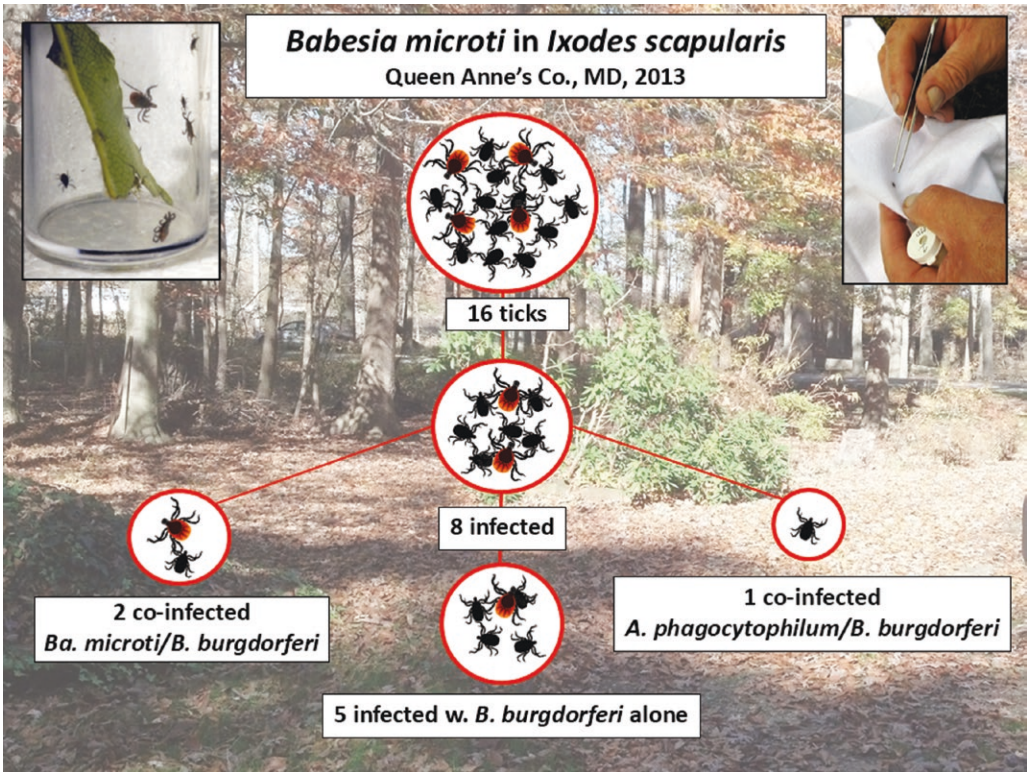
The range of *Babesia microti* (Franca, 1910)-infected ticks is expanding, resulting in locally acquired human babesiosis cases occurring in new areas: Maryland (2009), the District of Columbia (2013), Virginia (2016), and West Virginia (2017). We collected host-seeking ticks from old fields, ecotones, forested habitats and animal hosts in Delaware, Maryland, and Virginia, 2010 to 2024. *Ixodes scapularis* Say, the tick vector of babesiosis, was captured in all 3 states. PCR revealed *B. microti* in 2.7% (36/1310) of *I. scapularis*, with site prevalence ranging from <1% to 12.5% infected. The first *B. microti*-positive *I. scapularis* was collected in Northampton County, Virginia, 2012. Of the *B. microti*-infected ticks, 50% (18/36) were coinfected with *Borrelia burgdorferi* and one was triple-infected with *B. microti*, *B. burgdorferi*, and *Anaplasma phagocytophilum*. We collected *Ixodes keiransi* Beati, Nava, Venzal, and Guglielmone ticks from Delaware and Virginia. We found *B. microti* and *B. burgdorferi* in those from Virginia, and *B. burgdorferi* in ticks from a shrew in Delaware. To our knowledge, this is the first report of *B. microti* and *B. burgdorferi*-positive *I. keiransi* from Virginia, and the first report of *B. burgdorferi*-positive *I. keiransi* from Delaware. *Ixodes keiransi* ticks rarely bite humans but are involved in the maintenance and spread of pathogens when sympatric with *I. scapularis*. We tested a subset of both tick species for *Babesia duncani*; none were positive. Jurisdictions in the southern mid-Atlantic region should expect babesiosis cases, and Lyme disease and anaplasmosis coinfections, and healthcare providers should consider these tick-borne infections as part of the differential diagnosis.

Keywords: babesiosis, *Ixodes scapularis*, *Ixodes keiransi*, *Babesia microti*, *Borrelia burgdorferi*

Graphical Abstract



Introduction

Tick-borne diseases pose a major threat to human health in the United States, and tick range expansion is occurring at such a precipitous rate that public health guidance regarding tick-borne disease prevention and treatment can be rapidly rendered obsolete (Eisen and Paddock 2021). Human babesiosis is a potentially fatal illness caused in the eastern United States by infection with *Babesia microti* (Franca, 1910), a tick-borne protozoan parasite that is maintained through enzootic transmission cycles involving *Ixodes scapularis* Say, the blacklegged tick, and *Peromyscus leucopus* (Rafinesque), the white-footed mouse, among other vertebrate reservoirs.

Babesiosis was first reported from Nantucket Island, Massachusetts in 1969 and has become endemic in the northeastern United States in Connecticut, Maine, Massachusetts, New York, New Jersey, Rhode Island, and the upper midwestern states of Minnesota and Wisconsin (Swanson et al. 2023). The first autochthonous (locally acquired) case from Pennsylvania, in the mid-Atlantic region, was reported in 2011, however, babesiosis has not yet, as of 2025, been declared endemic in that state, although numbers of cases are increasing (Ingram and Crook 2020). Likewise, babesiosis is not considered endemic in the southern tier of mid-Atlantic states: Delaware, Maryland, Virginia, West Virginia, and the District of Columbia.

In 2009, local transmission, as defined in the epidemiological methods section below, of *B. microti* resulting in human infection was first identified in Maryland in Queen Anne's County, on the Delmarva Peninsula (Delaware and the Eastern Shores of Maryland and Virginia). This was the first autochthonous case reported from a state south of New Jersey. The patient was diagnosed by blood smear and PCR, and was also positive for infection with *Borrelia burgdorferi* (Wee et al. 2015) (Table 1). The patient was treated and recovered. Then in 2013, the first case of local transmission of *B. microti* was reported from the District of Columbia; in 2016, the first Virginia case was reported from the Eastern Shore of Virginia on the Delmarva peninsula; and the first West Virginia case was reported in 2017 (Table 1). In Delaware, there were 22 confirmed cases of babesiosis reported between 2015 and 2022, however, interviews were not conducted to determine if they were locally acquired (Delaware Health and Social Services, Division of Public Health, Office of Infectious Disease Epidemiology, data request, 28 Sept., 2023).

In nonendemic jurisdictions (eg Delaware, Maryland, Virginia, West Virginia, and the District of Columbia) clinicians might not be familiar with babesiosis, which can complicate diagnosis and delay treatment. Early symptoms of babesiosis are nonspecific, flu-like and overlap significantly with those of various bacterial and viral diseases, thus confounding prompt diagnosis. Clinicians who suspect

Table 1. Confirmed and probable autochthonous babesiosis cases, mid-Atlantic United States, 2009 to 2023

Jurisdiction	Year	No.	Region	Co-infection
District of Columbia, 2013 to 2023[a]	2013	1	N/A	None
	2014	2	N/A	None
	2015 to 2023	0	N/A	N/A
Maryland,	2009	1	Eastern Shore[c]	Lyme disease
2009 to 2022[b]	2010 to 2017	0	N/A	N/A
	2018	1	Baltimore Metro[d]	None
	2019	0	N/A	N/A
	2020	1	Baltimore Metro	Lyme disease
	2020	1	Eastern Shore	None
	2021	1	Eastern Shore	None
	2021	1	Baltimore Metro	None
	2022	1	Eastern Shore	None
	2022	1	Baltimore Metro	None
	2022	1	Baltimore Metro	Lyme disease
Virginia,	2016	1	Eastern Shore[f]	None
2016 to 2023[e]	2017	0	N/A	N/A
	2018	1	Eastern Shore	None
	2018	1	Eastern Shore	Ehrlichiosis or Anaplasmosis[g]
	2019	0	N/A	N/A
	2020	1	Fairfax[h]	None
	2020	1	Loudon[i]	None
	2021	1	Mount Rogers[j]	None
	2022	1	Eastern Shore	None
	2022	1	Mount Rogers	Lyme disease
	2022	1	Three Rivers[k]	None
	2022	1	New River[l]	None
	2023	1	Eastern Shore	Lyme disease
	2023	1	Eastern Shore	None
	2023	1	Eastern Shore	None
	2023	1	Fairfax	None
	2023	1	Loudon	None
	2023	1	Loudon	None
	2023	1	Central Virginia[m]	None
West Virginia,	2017	1	Pendleton County	None
2017 to 2023[n]	2018 to 2023	0	N/A	None

N/A, not applicable
[a]DC Department of Health, 2024.
[b]Maryland Department of Health, 2024.
[c]Eastern Shore Region includes Caroline, Cecil, Dorchester, Kent, Queen Anne's, Somerset, Talbot, Wicomico, Worcester Counties.
[d]Baltimore Metro Region includes Anne Arundel, Baltimore, Carroll, Harford, Howard Counties and Baltimore City.
[e]Virginia Department of Health, 2024.
[f]Eastern Shore District includes Accomack, Northampton Counties.
[g]Undetermined.
[h]Fairfax District includes Fairfax County and Fairfax City.
[i]Loudon District includes Loudon County.
[j]Mount Rogers District includes Bland, Carroll, Grayson, Smyth, Washington, Wythe Counties and Galax City.
[k]Three Rivers District includes Essex, Gloucester, King & Queen, King William, Lancaster, Mathews, Middlesex, Northumberland, Richmond, Westmoreland Counties.
[l]New River District includes Giles, Floyd, Montgomery, Pulaski Counties and Radford City.
[m]Central Virginia District includes Amherst, Appomattox, Bedford, Campbell Counties and Bedford City, Lynchburg City.
[n]West Virginia Department of Health, 2024.

a tick-borne illness might prescribe antibiotics, which while effective against many tick-borne bacterial pathogens, are ineffective against a parasitic infection like babesiosis. Delay in appropriate treatment for babesiosis for even 1 wk can contribute to disease severity including the potential for death (Herwaldt et al. 2003).

Ixodes scapularis is the vector of at least 7 human pathogens, including *B. microti*, *B. burgdorferi*, *Borrelia mayonii* (Lyme disease), *Anaplasma phagocytophilum* (anaplasmosis), *Borrelia miyamotoi* (hard tick relapsing fever), *Ehrlichia muris eauclairensis* (ehrlichiosis), and Powassan virus lineage II (Powassan virus disease) (Eisen and Eisen 2023). Genetic and behavioral analyses, environmental surveillance, and modeling have suggested that there are 2 variants of *I. scapularis*, termed northern and southern, coexisting in the mid-Atlantic United States (Norris et al. 1996, van Zee et al. 2015, Xu et al. 2020, Frederick et al. 2023).

Northern *I. scapularis* are frequently reported to bite humans, but in the southeast, reports of human biting are uncommon, therefore the northern populations are associated with much higher rates of human infection (Stromdahl and Hickling 2012). Differences in the questing behavior of nymphal and larval ticks is a key factor in the northern variant's ability to vector human pathogens. *Ixodes scapularis* nymphs from northern populations tend to quest more aggressively and higher on vegetation, whereas immature life stages of southern populations tend to remain on or below the leaf litter (Arsnoe et al. 2019). Furthermore, immature ticks of northern populations are more likely to feed on effective reservoirs of human pathogens (mice, shrews) compared to southern population immatures that feed on lizards, which are not effective reservoir hosts (Ginsberg et al. 2021). Consequently, nymphs from northern populations are more readily captured by flagging/dragging and are more likely to encounter humans and transmit pathogens (Arsnoe et al. 2019). In the first decades of the 21st century, the northern variant of *I. scapularis,* infected with *B. burgdorferi*, expanded swiftly and dramatically, bringing Lyme disease into areas where it was previously unknown—southern Michigan, Indiana, Ohio, and notably western Maryland, northern and western Virginia, and the District of Columbia (Johnson et al. 2017a, Eisen and Eisen 2023).

Establishment of *B. burgdorferi*-infected *I. scapularis* is a bellwether for the establishment of *B. microti*; as tick populations expand, the appearance of babesiosis cases tends to lag the appearance of Lyme disease cases (Diuk-Wasser et al. 2016). The competence of the key reservoir host *P. leucopus* for *B. microti* is increased by coinfection with *B. burgdorferi* (Dunn et al. 2014), therefore areas with established enzootic cycles of *B. burgdorferi* may be more permissive for invasion, establishment, and maintenance of *B. microti* (Diuk-Wasser et al. 2016). Ticks and animal hosts infected with *B. microti* are likely to be coinfected with *B. burgdorferi* (Diuk-Wasser et al. 2016). Likewise, Lyme disease and babesiosis coinfections regularly occur in human tick-bite victims and coinfected patients suffer from significantly more diverse and intense symptoms that persist longer than in patients infected with *B. burgdorferi* alone (Vannier and Krause 2012). Additionally, *B. microti* can be transmitted transplacentally from an infected female to offspring in both *P. leucopus* and *Microtus pennsylvanicus* (Ord) (the meadow vole, another important reservoir). Once introduced to a new geographic area, this route of vertical transmission likely contributes to the persistence of *B. microti* in the area (Tufts et al. 2023).

Ixodes keiransi Beati, Nava, Venzal, and Guglielmone (previously treated as North American populations of *Ixodes affinis*) is another tick infected at substantial rates with human pathogens. Although not known to bite humans frequently (Eisen 2022), it may play an important role in maintaining these pathogens in wildlife populations (Nadolny et al. 2011). Since the 1950s, the geographic range of *I. keiransi* has expanded north from Florida to Virginia (Nadolny and Gaff 2018) and in 2024 extends through New Jersey (Narvaez et al. 2024). Both *I. scapularis* and *I. keiransi* are well established in southeastern Virginia. *Ixodes scapularis* and *I. keiransi* can share the same hosts, therefore an increase in *B. microti* prevalence in *I. keiransi* could increase *B. microti* prevalence in *I. scapularis*, due to host sharing and co-feeding interactions (Nadolny et al. 2011). The introduction of *I. keiransi* into an area with sympatric populations of the northern variant of *I. scapularis* may drive amplification of the pathogens found in the ecosystem, and in doing so could increase the incidence of tick-borne disease in humans.

This paper is a collaborative effort among researchers and public health professionals who meet annually at the Mid-Atlantic Tick Summit (Nadolny et al. 2015), plus colleagues at the Centers for Disease Control and Prevention (CDC) and Mayo Clinic who kindly retested blinded samples. We report the results of PCR testing of *Ixodes* ticks field-collected in 4 independent studies in locations where cases of babesiosis were beginning to appear, but where the pathogen had not yet been detected in ticks: (i) Kent, New Castle and Sussex Counties, Delaware (DE), (ii) Aberdeen Proving Ground, Harford County, Maryland (APG), (iii) Queen Anne's County, Maryland (QAC), and (iv) southeastern Virginia and the Virginia Eastern Shore on the Delmarva Peninsula (ODU), Additionally, we tested a subset of these tick samples for *Babesia duncani*, a tick-borne protozoan parasitic agent of human disease that is found in *Ixodes pacificus* ticks exclusively in western North America, because there have been numerous reports from commercial and private laboratories of positive serologies for *B. duncani* in humans from outside the range of *I. pacificus* (Prince et al. 2010, Stromdahl and Hickling 2012). We also summarize the number and map the location of autochthonous cases of human babesiosis investigated and reported by DC Health (District of Columbia), the Maryland Department of Health, the Virginia Department of Health and the West Virginia Department of Health and Human Resources, and we map the location of the *B. microti*-positive ticks from our 4 collections, plus additional *B. microti*-positive ticks from Maryland and Virginia reported in previous publications.

Methods

Epidemiological Methods

Laboratories and healthcare providers are required by state code to submit babesiosis laboratory reports to the local health department within 1 wk of the test. In the District of Columbia, providers must submit a case report to DC Health within 48 h of a provisional diagnosis or appearance of suspicious symptoms, and laboratory test results should follow as soon as available. Local health departments investigate suspected human babesiosis cases for patient demographics, clinical symptoms, epidemiological exposures, and public health action initiated by public health. Exposure history collected includes travel history, exposure to ticks and tick habitat, occupational exposure, and blood transfusion and organ transplant history. We considered cases to be autochthonous, or locally acquired, when there was no indication of travel, transfusion or organ transplant history for the case.

Tick Collection and Identification

We collected questing ticks by flagging and dragging (Sonenshine 1993, Rulison et al. 2013) in old fields, ecotones and forested habitats at sites in Queen Anne's County, Maryland, 2013 (QAC); southeastern Virginia and the Virginia Eastern Shore, 2010 to 2017 (ODU); Aberdeen Proving Ground, Harford County, Maryland, 2017 to 2019, 2022 to 2023 (APG); and Kent, New Castle, and Sussex Counties, Delaware, 2019 to 2024 (DE). Additionally, we collected ticks from small mammal hosts trapped in Sherman live traps (H.B. Sherman Traps, Tallahassee, FL) baited with oats and peanut butter in Sussex Co., Delaware, in 2023, in accordance with Delaware Division of Fish and Wildlife permit no. 2023-WSC-032. Ticks were identified morphologically using taxonomic keys (Clifford et al. 1961, Keirans and Litwak 1989, Durden and Keirens 1996).

DNA Isolation and PCR

Genomic tick DNA was isolated using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Frederick, MD) or the Fermentas GeneJet DNA extraction kit (ThermoFisher Scientific, Waltham, MA). DNA of individual ticks collected at QAC and APG were screened using PCR for *B. microti, A. phagocytophilum* and *B. burgdorferi* sensu stricto. Those collected from the ODU sites were tested for *B. microti*, positives in this PCR were also screened for *B. burgdorferi* sensu stricto, and a subset of 45 samples were screened for *B. duncani*. Nymphs and adults from DE were screened for *B. microti*, *A. phagocytophilum*, and *B. burgdorferi* sensu stricto. Samples from QAC and APG positive in the initial screen for *B. microti* were confirmed by a second PCR for a different genetic target. QAC samples positive for *B. microti* were also sent blinded for additional PCR confirmation to the CDC Division of Parasitic Diseases and Malaria in Atlanta, Georgia (CDC) and to the Mayo Clinic, Rochester, Minnesota. Samples from ODU (1 *I. keiransi,* 1 *I. scapularis*) and DE (13 *I. scapularis*) positive in the initial screen for *B. microti* were sequenced to confirm *B. microti* identity. PCR primers are listed in Table 2.

PCR Protocols

Aberdeen Proving Ground, MD (APG)

Multiplex PCR for *B. burgdorferi* (gB31 gene), *A. phagocytophilum* (*msp4* gene), and *B. microti* (18S gene) was performed using the M2 primer set and protocols from Hojgaard et al. (2014). Any samples positive for *A. phagocytophilum* were reconfirmed using primers targeting the *gltA* gene and protocols from Henningsson et al. (2015), those positive for *B. microti* were confirmed using primers for a different region of the 18S rRNA gene and protocols from Rollend et al. (2013), and those positive for *B. burgdorferi* were reconfirmed with *fliD* gene primers and protocols from Johnson et al. (2017b).

Kent, New Castle, and Sussex Counties, Delaware (DE)

PCR targeting *B. burgdorferi*, *A. phagocytophilum*, and *B. microti* was performed using the M2 primer set for the gB31 gene and protocols from Hojgaard et al. (2014).

Southeastern Virginia and the Virginia Eastern Shore (ODU)

In the years 2010 to 2012 and 2015 to 2017, extracted DNA was screened for the presence of *B. microti* using a singleplex paired real-time PCR assay with primers and probe (FAM-labeled) and protocols from Graham et al. (2018). In 2013 and 2014 extracted DNA was screened for *B. microti* using the paired multiplex TaqMan real-time PCR assay described by Graham et al. (2018).

A subset of 45 samples were screened for *B. duncani* DNA presence using primers and protocols previously described in Wilson et al. (2015).

Queen Anne's County, MD (QAC)

Babesia microti PCR was performed using a real-time assay targeting 18S rRNA of *B. microti* (Tonnetti et al. 2009). Any samples positive were tested again using conventional primers BAB 1 and BAB 4 targeting the 18S rRNA ss-rDNA gene (Persing et al. 1992). PCR for *A. phagocytophilum* was first done using a melting curve analysis of amplification of the groESL gene which differentiates *A. phagocytophilum*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Ehrlichia muris eauclairensis*, and *Ehrlichia* sp. "Panola Mountain" (Bell and Patel 2005). Any samples positive for *A. phagocytophilum* in this assay were tested again in a nested PCR using primers for the 16S rRNA gene of *A. phagocytophilum* (Massung et al. 1998). Samples positive for *B. microti* were then sent blinded to CDC where they were tested using the nested array of primers BAB 1, BAB 2, BAB 3, and BAB 4 from Persing et al. (1992). These samples were then further confirmed at Mayo Clinic using a real-time multiplex PCR designed to detect and differentiate targeted *Babesia* species: *Babesia divergens*/*B. divergens*-like organism (eg MO-1 strain), *B. duncani*, and *B. microti*. *Borrelia burgdorferi* PCR was performed using primers and a probe designed to anneal to the ospA gene of *B. burgdorferi* (Straubinger 2000). Any samples positive in this assay were tested again in a PCR for targeting the inner part of the *fla*

Table 2. PCR primers used for detection of pathogens in ticks

Survey	Pathogen	Gene	Reference
QAC	*Anaplasma phagocytophilum*	*groEL* (screen)	Bell and Patel (2005)
		16S rRNA (confirm)	Massung et al. (1998)
	Borrelia burgdorferi sensu stricto	OspA (screen)	Straubinger (2000)
	Borrelia burgdorferi sensu lato	*fla* (confirm)	Leutenegger et al. (1999)
	Babesia microti	18S rRNA (screen)	Tonnetti et al. (2009)
		18S rRNA (confirm)	Persing et al. (1992)
		18S rRNA (confirm)	Burgess et al. (2017)
APG	*Anaplasma phagocytophilum*	*msp4* (screen)	Hojgaard et al. (2014)
		gltA (confirm)	Henningsson et al. (2015)
	Borrelia burgdorferi	gB31 (screen)	Hojgaard et al. (2014)
		fliD (confirm)	Johnson et al. (2017b)
	Babesia microti	18S rRNA (screen)	Hojgaard et al. (2014)
		18S rRNA (confirm)	Rollend et al. (2013)
ODU	*Babesia microti*	18S rRNA	Graham et al. (2018)
		sa1	Graham et al. (2018)
	Babesia duncani	ITS	Wilson et al. (2015)
DE	*Anaplasma phagocytophilum*	*msp4* (screen)	Hojgaard et al. (2014)
		gltA (confirm)	Henningsson et al. (2015)
	Babesia microti	18S rRNA (screen)	Graham et al. (2018), Hojgaard et al. (2014)
		18S rRNA (confirm)	Casati et al. (2006)
	Borrelia burgdorferi	gB31 (screen)	Hojgaard et al. (2014)
		fla (confirm)	Leutenegger et al. (1999)

QAC, Queen Anne's County, Maryland; ODU, Old Dominion University, Virginia; APG, Aberdeen Proving Ground, Maryland; DE, Delaware.

Table 3. *Babesia microti* infection and *Borrelia burgdorferi* coinfection in *Ixodes* spp. ticks field collected in surveys across Delaware, Maryland, and Virginia, 2010 to 2024

			No. pos./no. tested (% pos.)			
			I. scapularis		I. keiransi	
Study	Years	Stage	*Bm*	*Bb + Bm*[a]	*Bm*	*Bb + Bm*
QAC[b]	2013	Adult	2/16 (12.5)	2/2 (100.0)	NC	NC
ODU[b]	2010 to 2017	Adult	1/337 (0.3)	1/1 (100.0)	5/628 (0.8)	4/5 (80.0)
		Nymph	2/306 (0.65)	1/2 (50.0)	0/1 (0)	0
APG	2017 to 2023	Adult[c]	1/1 (100.0)	1/1 (100.0)	NC	NC
		Nymph	17/150 (11.3)	6/17 (35.3)	NC	NC
DE[b]	2019 to 2024	Adult	8/342 (2.3)	4/8 (50.0)	0/14 (0)	0
		Nymph	5/158 (3.2)	3/5 (60.0)	0/2 (0)	0
Total	2010 to 2023	Adult	12/696 (1.7)	8/12 (66.7)	5/642 (0.8)	4/5 (80.0)
		Nymph	24/614 (3.9)	10/24 (41.7)	0/3(0)	0

QAC, Queen Anne's County, Maryland; ODU, Old Dominion University, Virginia; APG, Aberdeen Proving Ground, Maryland; DE, Delaware; *Bb*, *Borrelia burgdorferi*; *Bm*, *Babesia microti*; NC, none collected.

[a]No. *Bb* pos./no. *Bm* pos.

[b]All *Babesia microti* positive QAC ticks, a subset of ODU ticks and a subset of DE ticks were tested for *Babesia duncani*, none were positive.

[c]This tick was also positive for *Anaplasma phagocytophilum* (triple-infected).

gene (Leutenegger et al. 1999). Details of all the PCR methods listed above are published in (Stromdahl et al. 2014).

Sequence Confirmation *Babesia microti*-Positive Samples

Southeastern Virginia and the Virginia Eastern Shore (ODU)

Samples positive for *B. microti* in real-time assays were amplified using endpoint PCR targeting a 425 bp fragment in the 18S rRNA gene. Amplification was carried out in 20 µl reactions with 10 µl of 2X EconoTaq PLUS master mix (Lugien Corp., Middleton, WI), 1 µl of each primer (BN2 and BJ1; 10 µM conc.), 3 µl of nuclease-free water, and 5 µl of DNA template using the conditions outlined (Casati et al. 2006). Samples were visualized using gel electrophoresis and purified using the Wizard PCR preps DNA Purification System (Promega, Madison, WI) according to the manufacturer's instructions. Sequencing reactions were performed using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Sequence data was assembled and curated using Geneious (https://www.geneious.com) and compared with known sequences using NCBI BLAST (http://blast.ncbi.nlm.nih.gov).

Kent, New Castle, and Sussex Counties, Delaware (DE)

Positive samples for *B. microti* in real-time assays were amplified using endpoint PCR targeting a 425 bp fragment in the 18S *rRNA* gene (Casati et al. 2006). Amplification was performed in 50 µl samples contained 25 µl of Promega GoTaq Green Master Mix (Promega, Madison, WI), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 2 µl of sample tick gDNA and 21 µl of Nuclease-Free Water. Samples were visualized using gel electrophoresis. The post-PCR product was cleaned using the Promega ReliaPrep DNA Clean-Up and Concentration System (Promega, Madison, WI), then sequenced using the Applied Biosystems SeqStudio Genetic Analyzer. Sequencing reactions were then performed using the BigDye Terminator v3.1 Cycle Sequencing Kit. Subsequent.ab1 sequence data was uploaded to Snapgene (http://www.snapgene.com) software, contigs were created, and the sequence was verified via NCBI BLAST.

Results

Queen Anne's County (QAC)

In November 2013, we collected 16 questing *I. scapularis* adults in the yard and a nearby site of the first Maryland patient confirmed to have autochthonous babesiosis (Wee et al. 2015). *Borrelia burgdorferi* was detected in 8 of 16 ticks; 2 of these 16 were coinfected with *B. microti*, and 1 with *A. phagocytophilum*. *Babesia microti* infection of the 2 positive tick samples was confirmed by additional PCR at CDC Parasitic Diseases and Malaria Division and at the Mayo Clinic. *Babesia duncani* was not detected (Table 3 and Fig. 1B). Nymphal ticks were inactive in the fall season when we collected, however, adults can provide a good indication of the local pathogen pool because they are likely to have taken an infected bloodmeal as nymphs on a resident, non-migratory host (Walk et al. 2009). To the authors' knowledge, at that time *B. microti* had not been detected in *I. scapularis* from Maryland (Stromdahl and Hickling 2012).

Aberdeen Proving Ground, MD (APG)

From 2017 to 2023, we collected questing *I. scapularis* (150 nymphs and 1 female) during May and June from 7 sites in the ordnance testing ranges of APG, Maryland, at the mouth of the Bush River on the western shore of the Chesapeake Bay. Of these, 17 nymphs (17/150 = 11.3%) and the female were positive for *B. microti*. Of the 17 *B. microti*-positive nymphs, 6 were coinfected with *B. burgdorferi*, and the female was triple-infected with *A. phagocytophilum*, *B. microti*, and *B. burgdorferi*. The 18 *B. microti*-positive ticks were collected from 5 sites across the APG ranges and throughout all years of the study (Table 3 and Fig. 1B).

Southeastern Virginia and the Virginia Eastern Shore (ODU)

From 2010 to 2017, we collected questing *Ixodes* sp. (629 *I. keiransi* and 643 *I. scapularis*) from 17 sites in southeastern Virginia and 1 site on the Virginia Eastern Shore on the Delmarva Peninsula. The first ticks positive for *B. microti* were collected in 2012. *Babesia microti* was detected in 5 *I. keiransi* adults (5/628 = 0.8%) and 3 *I.*

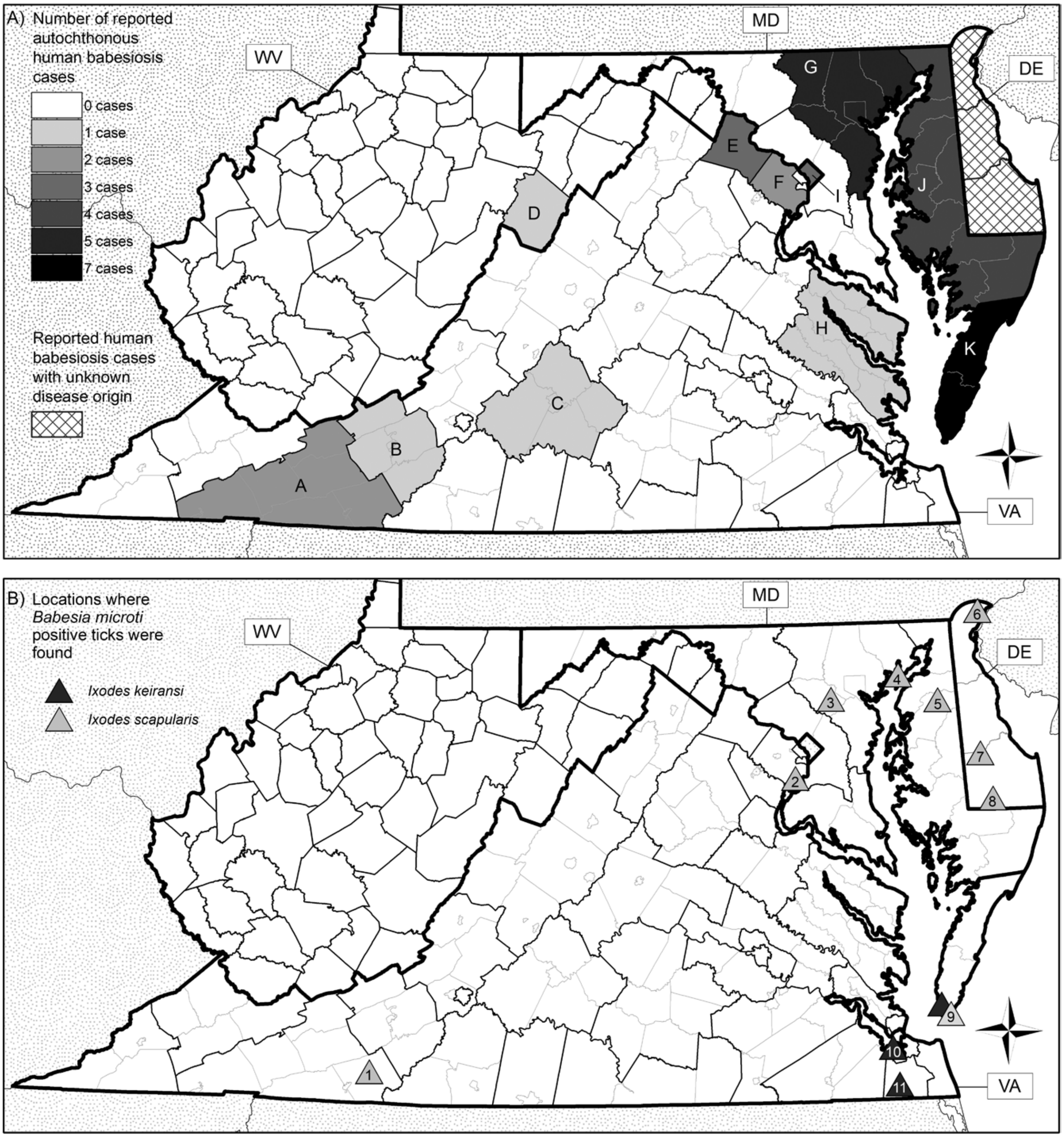


Fig. 1. A) Locations of autochthonous cases of human babesiosis in Delaware (DE), Maryland (MD), Virginia (VA), and West Virginia (WV). Health jurisdictions which reported autochthonous cases, 2009 to 2023, are shaded in gray (the darker the gray, the more cases) and are labeled as follows: A) Mt. Rogers Health District, VA; B) New River Health District, VA; C) Central Virginia Health District, VA; D) Pendleton Co., WV; E) Loudon Health District, VA; F) Fairfax Health District, VA; G) Baltimore Metro Region, MD; H) Three Rivers Health District, VA; I) District of Columbia; J) Eastern Shore Region, MD; K) Eastern Shore Health District, VA. In DE, 22 confirmed cases of babesiosis reported were between 2015 and 2022, however, interviews were not conducted to determine if they were locally acquired. B) Collection sites of *Ixodes* ticks PCR-positive for *Babesia microti* in DE, MD, and VA. Numbered triangles (light gray, *Ixodes scapularis*; dark gray, *Ixodes keiransi*) identify the locations of tick collection, 2010 to 2024. (1) Carroll Co., VA; (2) Fairfax Co., VA; (3) Howard Co., MD; (4) Harford Co., MD; (5) Queen Anne's Co., MD; (6) New Castle Co., DE; (7, 8), Sussex Co., DE; (9), Northampton Co., VA; (10) City of Portsmouth, VA; (11) City of Chesapeake, VA.

scapularis (3/643 = 0.47%; 1/337 [0.3%] adults and 2/306 [0.65%] nymphs). All 3 of the *B. microti*-positive *I. scapularis* and 3 of the 5 positive *I. keiransi* were all collected in Northampton County on the Virginia Eastern Shore; coinfection with *B. burgdorferi* was detected in 2 of these 3 *B. microti*-positive *I. scapularis* and all 3 *B. microti*-infected *I. keiransi*. One *B. microti*-positive *I. keiransi* was found in the City of Portsmouth, Virginia and 1 coinfected with *B. burgdorferi* and *B. microti* was found in the City of Chesapeake, Virginia (Table 3 and Fig 1B).

Two of the *B. microti* positive samples, 1 *I. scapularis* and 1 *I. keiransi*, were confirmed by 18S sequence analysis which showed a >99% match over a 383 to 443 bp fragment to *B. microti* (Accession

Table 4. Pathogen testing of *Ixodes scapularis* (*Ixodes dammini*) ticks collected from the Delmarva Peninsula, 1980 to 2019. From 1979 to 1992, the northern population of *I. scapularis* was considered a separate species, *I. dammini.* Bm, *Babesia microti;* Bb, *Borrelia burgdorferi;* Ap, *Anaplasma phagocytophilum;* Bmiya, *Borrelia miyamotoi.*

Year	Location	Ticks	Tested for/detected?	Testing method	Reference
1980 to 1981	Assateague Island, MD	*I. dammini*	Bm/no	Microscopy	Coan and Stiller (1986)
1987	Assateague Island, MD	*I. dammini*[a]	Spirochetes/yes	Microscopy, IFA	Ginsburg (1992)
1987	Parramore Island, VA	*I. dammini*[a]	Bb/yes	IFA	Levine et al. (1991)
1987 to 1989	Assateague Island, MD, VA	*I. scapularis*[a]	Bb/yes	IFA	Oliver et al. (1999)
1988	3 counties, DE[b]	*I. scapularis*	Bb/yes	Not reported	Wolfe et al. (1994)
1989	9 counties, MD[c]	*I. dammini*	Bb/yes	DFA	Amerasinghe et al. (1992)
1991	8 counties, MD[d]	*I. dammini*	Bb/yes	DFA	Amerasinghe et al. (1993)
1991 to 1994	Assateague Island, VA	*I. scapularis*[a]	Bb/yes	IFA	Sonenshine et al. (1995)
1994	Assateague Island, VA	*I. scapularis*	Bb/yes	IFA	Casteel and Sonenshine (1996)
1998	3 counties, DE[b]	*I. scapularis*	Bb/yes, Ap/yes	PCR	Curran et al. (2000)
2003	8 counties, MD[e]	*I. scapularis*[a]	Bb/yes, Ap/yes, Bm/no	PCR	Swanson and Norris (2007)
2013, 2014	New Castle county, DE	*I. scapularis*[a]	Bb/yes, Ap/yes, Bm/no, Bmiya/yes	PCR	Adalsteinsson et al. (2018)
2019	3 counties, DE[b]	*I. scapularis*[a]	Bb/yes	PCR	Shifflett et al. (2023)

[a]Including nymphs collected from vegetation.
[b]New Castle, Kent, Sussex Counties.
[c]Cecil, Queen Anne's, Talbot, Kent, Worcester, Somerset, Wicomico, Caroline, Dorchester Counties.
[d]Cecil, Queen Anne's, Talbot, Kent, Worcester, Wicomico, Caroline, Dorchester Counties.
[e]Queen Anne's, Talbot, Kent, Worcester, Somerset, Wicomico, Caroline, Dorchester Counties.

numbers: XR_002459986 & KC821597). Of the 1,278 samples screened for *B. microti*, 58 (54 *I. keiransi* and 4 *I. scapularis* adults) were also tested for the presence of *B. duncani;* no positive samples were detected.

Kent, New Castle, and Sussex Counties, Delaware (DE)

We screened questing *I. scapularis* (158 nymphs and 342 adults) collected from 2019 to 2023 in all 3 counties of Delaware for *B. microti* and 5/158 (3.2%) nymphs and 8/342 (2.3%) adults were positive. The first *B. microti*-positive tick was collected in 2019. *Borrelia burgdorferi* coinfection was identified in 3/5 *B. microti*-positive nymphs and 4/8 *B. microti*-positive adults. Sequence analysis showed that all 13 *B. microti*-positive samples were a >99% match to a 422 to 449 bp fragment of the *B. microti* 18S ribosomal RNA gene (Accession within location 465-979. number: AF231348).

A questing nymphal *I. keiransi* tick was collected from vegetation in New Castle County in April 2021. Additional specimens of *I. keiransi* (12 female, 7 males, 1 nymph) were also collected from vegetation in all 3 counties of Delaware between 2022 and 2024. Of these, 16 were screened for pathogens; none were positive. Additionally, 5 *I. keiransi* (1 larva, 4 nymphs) were collected from one *Blarina brevicauda* (Say) (northern short-tailed shrew) in Sussex County in October 2023. The larva and 3 nymphs from the shrew were screened for pathogens; the 3 nymphs were positive for *B. burgdorferi*. These specimens represent a record for statewide distribution of *I. keiransi* in Delaware.

Discussion

Delmarva Peninsula Hotspot

The Delmarva Peninsula was a transmission hotspot (an area of higher risk of disease acquisition; Lessler et al. 2017)) for autochthonous cases of babesiosis in the southern mid-Atlantic region, 2009 to 2023. Seven of 17 (41.2%) Virginia cases were reported from the Virginia Eastern Shore Health District (Table 1 and Fig. 1A) and 4 of 9 (44.4%) Maryland cases were reported from the Maryland Eastern Shore Region (Table 1 and Fig. 1A). Of the 30 total autochthonous cases from Virginia, Maryland, West Virginia, and the District of Columbia combined, 37.6% (11/30) were from the Delmarva jurisdictions (Table 1 and Fig. 1A). *Babesia microti*-positive *I. scapularis* ticks were found at the DE, ODU, and QAC collection sites throughout the Delmarva Peninsula (Fig. 1B). The first *Babesia microti*-positive *I. scapularis* reported from Virginia were collected in 2012 in Northampton County, Virginia, Delmarva Peninsula and the first reported from Maryland were collected in 2013 in Queen Anne's County, Maryland, Delmarva Peninsula. This tick distribution further substantiates the Delmarva Peninsula as a hotspot for autochthonous human cases of babesiosis.

Baltimore Metro Health District Hotspot

The other hotspot for autochthonous human cases of babesiosis was the Baltimore Metro Health District (Anne Arundel, Baltimore, Carroll, Harford, Howard Counties, and Baltimore City) in eastern Maryland on the western shore of the Chesapeake Bay, adjacent to the Delmarva Peninsula (Table 1 and Fig. 1A). Five of 9 (55.5%) Maryland cases were reported from the Baltimore Metro Health District, 2018 to 2022. *Babesia microti* first appeared at approximately the same time, reported in a *Peromyscus* sp. mouse and in a small number of *I. scapularis* collected from deer and from the environment during 2016 and 2017, in Howard County, Maryland (Milholland et al. 2021a, b). Of the 18 questing *I. scapularis* adults tested in this study, 4 were positive *for B. microti* alone, 14 were coinfected with *B. microti* and *B. burgdorferi*, 1 was triple-infected with *B. microti*, *A. phagocytophilum*, and *B. burgdorferi*, and 1 was triple-infected with *B. microti*, *B. miyamotoi*, and *B. burgdorferi*. Additionally, *B. microti*-positive *I. scapularis* (18/151 (11.9%) were field collected in our APG study, 2017 to 2023, and in 2020, *B. microti* and *B. burgdorferi* coinfection was detected in an *I. scapularis* removed from a human in Harford County through the MilTICK tick testing program. Prior to this, *B. microti* had not been found in 1,587 *I. scapularis* submitted from Maryland, Delaware, Virginia or West Virginia to the MilTICK program, 2002 to 2020. Then in 2024, another *I. scapularis* from a human submitted to

MilTICK from Harford County was found positive for *B. microti* and *A. phagocytophilum* (VectorMap, 2024) (Fig. 1B).

Natural History of *I. scapularis, B. burgdorferi*, and *B. microti* in the Hotspots

Finding a relatively robust infection rate of *B. microti* (2/16 = 12.5%) in *I. scapularis* collected in 2013 near the home of the index patient in Queen Anne's County, Maryland, Delmarva Peninsula, and in 18/151 (11.9%) of *I. scapularis* collected from a small area of APG, Baltimore Metro Region, Maryland, suggests populations of *I. scapularis* in those locations may be long-established (Diuk-Wasser et al. 2014) (Table 3). Furthermore, the frequency of *B. microti*-infected ticks from these hotspots coinfected with *B. burgdorferi* was >10% (Table 3), which is consistent with pathogen infection prevalences of *I. scapularis* from long-endemic regions (Diuk-Wasser et al. 2016, Johnson et al. 2017a).

Multiple sources of evidence indicate that *I. scapularis* has been established in coastal Maryland and Virginia, and on the Delmarva Peninsula since the 1980s, perhaps much longer. An ecological niche model projected on Last Glacial Maximum climate reconstructions suggests that the northern *I. scapularis* population was derived from a small refuge just south of the Pleistocene ice sheet on the Atlantic continental shelf off the current Delmarva Peninsula (Xu et al. 2020). *Ixodes scapularis* has been recorded from eastern Maryland and coastal Virginia since the beginning of the 20th century (Eisen and Eisen 2023). Since 1987, *I. scapularis* infected with *B. burgdorferi* have been reported from the Delmarva, including nymphs swept from vegetation (Table 4). Infection with *B. burgdorferi* in field-collected, questing nymphal *I. scapularis* indicates the potential for human disease transmission and is characteristic of the northern variant (Arsnoe et al. 2019), and infection of these ticks with *B. burgdorferi* presages infection with *B. microti*.

This pattern of spatial distribution of *B. burgdorferi* infection also appears in an ingenious investigation of preserved mammal host skins (*P. leucopus*) collected between 1911 and 2000 from sites throughout Virginia and North Carolina. *Borrelia burgdorferi* (presumably vectored by *I. scapularis*) was detected only in samples from Northampton County, Virginia, Delmarva Peninsula, collected in 1989 (Leber et al. 2022). At this time, *I. keiransi* had not yet invaded Virginia (Nadolny et al. 2011).

Accordingly, *B. microti* infection was found only in *I. scapularis* collected at the ODU site on the Eastern Shore of Virginia, not in *I. scapularis* from the 17 southeastern Virginia sites (Fig 1B). Furthermore, publications describing cases of Lyme disease in Virginia from 1986 to 2014 report fewer cases of Lyme disease in the southeastern counties than from those on the Eastern Shore (Heimberger et al. 1990, Brinkerhoff et al. 2014, Li et al. 2014, Lantos et al. 2015). Fewer Lyme disease cases in the southeastern counties suggests that humans in southeastern Virginia do not encounter the northern variant of *I. scapularis*. Differences in frequency of *B. burgdorferi* infection (a precursor of *B. microti* infection) suggests that the northern variant of *I. scapularis* was active on the Delmarva Peninsula but not southeastern Virginia.

Lyme disease cases have been reported from eastern Maryland since the 1980s (Thambidurai et al.1988, Heimberger et al. 1990). Lyme disease was first reported from Delaware in 1989 (Wolfe et al. 1994) and has an increasing incidence rate (Curran et al. 2000, Gupta et al. 2018). *Ixodes scapularis* ticks were reported from Harford County, Baltimore Metro Region, in the 1960s (Coan and Stiller 1986) and *B. burgdorferi*-infected *I. scapularis* have been collected in eastern Maryland since surveillance for the vector of Lyme disease began in the 1980s (Amerasinghe et al.1993, Fleshman et al. 2022), perhaps because white-tailed deer (*Odocoileus virginianus* (Zimmermann)), the reproductive host of *I. scapularis*, were re-established there early in the 20th century. In the 1930s, APG, in Harford County, was a site for restocking white-tailed deer, and the species continues to flourish there (MDNR, 2020).

District of Columbia and Northern Virginia

The first autochthonous case of babesiosis in the District of Columbia dates from 2013 (Table 1). *Ixodes scapularis* was first reported from the District in collections from 2014 and 2015 and pathogen testing of 253 questing nymphs did not reveal *B. microti* infection, however, >20% were infected with *B. burgdorferi* (Johnson et al. 2017a), the precursor of *B. microti* infection.

Borrelia microti-positive *I. scapularis* were first collected in northern Virginia at approximately the same time as autochthonous cases began to appear. In 2022, *B. microti* and *B. burgdorferi* coinfection was detected by the MilTICK Program in an *I. scapularis* removed from a human in Fairfax County, Virginia (VectorMap, 2024) (Fig. 1B), where autochthonous cases have been reported from Fairfax (2020) and Loudon (2020, 2023) County Health Districts (Fig. 1A).

Published surveillance suggests that *I. scapularis* has invaded the District of Columbia and northern Virginia more recently than coastal regions and the Delmarva Peninsula. The southward range expansion of *I. scapularis* into northern Virginia is illustrated in county distribution maps of ticks from 1996, 2015, and 2022 (Eisen and Eisen 2023, Fig. 3). *Ixodes scapularis* began to appear in northern Virginia at the turn of the 21st century (Dennis et al. 1998), and within 2 decades, *B. burgdorferi* infection in these ticks (Fleshman et al. 2021, 2022) and cases of Lyme disease (Brinkerhoff et al. 2014) had become common, but *B. microti* infection was still absent or rare, although surveillance for *B. microti* was also absent or rare. *Babesia microti* was not reported from the counties of northern Virginia in survey of county-level distribution of *I. scapularis* pathogens 2004 to 2021 (Fleshman et al. 2022), nor from all of Virginia in a survey of pathogen testing records 2004 to 2022 of host-seeking *I. scapularis* adults and nymphs collected in the national tick surveillance effort (Foster et al. 2023).

Western Virginia

The first case of autochthonous babesiosis from western Virginia was recognized in 2021 from the Mt. Rogers Health District, followed by a second case in 2022, and another from the adjacent New River Health District, also in 2022 (Table 1). The first *B. microti*-positive *I. scapularis* nymph was collected in 2019 in Carroll County, Va., Mt. Rogers Health District, it was also positive for *B. burgdorferi* (Fleshman et al. 2022) (Fig. 1B).

Published surveillance also suggests that western Virginia might be even more recently colonized by *I. scapularis* than northern Virginia. This tick was not reported from western Virginia in a comprehensive national survey of collection records published in 1998 (Dennis et al.1998), nor collected in a large field survey conducted 2004 to 2006 across the eastern United States targeting nymphal *I. scapularis* (Diuk-Wasser et al. 2010), and cases of Lyme disease in these regions were rare until 2007 when a steep uptick of Lyme disease cases was reported from counties along the Appalachian Mountains in the western part of Virginia (Lantos et al. 2015).

In reaction to the unexpected rise in cases of Lyme disease, tick surveys were undertaken in Virginia which revealed that *I. scapularis* infected with *B. burgdorferi* had expanded into these areas where it had been historically absent (Brinkerhoff et al. 2014). Investigators then surveyed additional western areas in Virginia and were able to collect nymphal ticks by sweeping vegetation, indicating the questing behavior characteristic of northern population *I. scapularis*, and 3 of these tick collection studies reported infection with *B. burgdorferi* (Herrin et al. 2014, Jackson 2020, Whitlow et al. 2022). Evidence from tick genetic analysis studies also suggested that the ticks advancing in western Virginia were part of the northern population (Kelly et al., 2014, Jackson, 2020, Xu et al. 2020, Ghosh et al. 2021, Frederick et al. 2023). None of these investigations included pathogen analysis for *B. microti*.

West Virginia

Babesia microti has not yet (as of 2025) been detected in ticks in West Virginia (Eric Dotseth, unpublished data), however, the first case of autochthonous babesiosis was reported in 2017 from Pendleton County, near the Virginia border (WV DHHR, 2017) (Table 1 and Fig. 1A). West Virginia has also experienced a rapid invasion of *B. burgdorferi*-infected *I. scapularis*. In 2010, the only Lyme disease endemic counties were in the eastern panhandle (WV DHHR, 2010), and the first questing *I. scapularis* were collected there in 2011, although no pathogens were detected (WV DHHR, 2011). In 2012, *I. scapularis* positive for *B. burgdorferi* and *A. phagocytophilum* were collected (WV DHHR, 2012), and by 2021, every county in the state had reported *I. scapularis* and cases of Lyme disease (WV DHHR, 2021).

Ixodes keiransi

We also collected *I. keiransi* at the ODU and DE sites (Fig. 1B). *Babesia microti* and *B. burgdorferi* coinfection was detected in questing *I. keiransi* from several ODU sites in Virginia (Northampton County, City of Portsmouth, City of Chesapeake). Additionally, *B. burgdorferi* (precursor of *B. microti* infection) was detected in *I. keiransi* removed from a shrew in Sussex County, Delaware. Because these ticks were feeding on the shrew host, it cannot be determined if the detection of *B. burgdorferi* represents an active infection in the tick, or borreliae taken up in the host bloodmeal. However, this finding suggests that *B. burgdorferi* is circulating in *I. keiransi* in Delaware, because *I. keiransi* is an established vector of *B. burgdorferi* in rodent cycles (Oliver et al. 2003).

Ixodes scapularis is a recognized vector for *B. microti*, however, the pathogen has not previously been detected in *I. keiransi*. The finding of *B. microti* in *I. keiransi* raises important questions for the potential amplification of the pathogen in the sylvatic cycle similar to the implications found for *B. burgdorferi* with the range expansion of *I. keiransi* (Nadolny et al. 2011). The mid-Atlantic states may be an important expansion front not only for *B. microti*-infected *I. scapularis*, but also for *I. keiransi*. In southeastern Virginia and on the Delmarva Peninsula *I. scapularis* and *I. keiransi* are sympatric and may be co-involved in the maintenance of the *B. microti* sylvatic cycle. Of the 8 *B. microti*-positive ticks found in the ODU study, 62.5% of them were *I. keiransi*, suggesting this tick may more easily acquire *B. microti* than *I. scapularis*. Other research has reported a greater prevalence of *B. burgdorferi* infection in *I. keiransi* as compared to *I. scapularis* (Oliver et al. 2003, Maggi et al. 2010, 2019). The sympatry of the two-tick species may facilitate the establishment of *B. microti* in populations of the northern variant of *I. scapularis* as *I. keiransi* moves north.

Babesia duncani

We detected no positive samples in the subsets of ticks that were tested for *B. duncani*. This was expected because *B. duncani* DNA has never been amplified from a tick or mammal host east of the Rocky Mountains. *Babesia duncani* may be identified in human samples because of serological cross-reactivity with *Babesia odocoilei* (Scott et al. 2021, Maggi et al. 2024), a tick-borne protozoan parasite of undetermined epidemiological importance (Guillot et al. 2024). *Babesia odocoilei* causes disease in white-tailed deer and other cervids, and is found in *I. scapularis* ticks throughout their range typically at greater prevalences than *B. microti* (Perry et al. 1985, Armstrong et al. 1998, Shoelkopf et al. 2005, Zemsch et al. 2021).

Conclusions

Cases of babesiosis in the southern mid-Atlantic region are increasing (Fig. 2). Healthcare providers, public health practitioners, tick-bite victims, and the general public need to be aware of this emerging human health threat. Babesiosis can be severe in the elderly or immunocompromised (Vannier and Krause 2012), especially when patients have concurrent infections with *B. burgdorferi*. Diagnosis can be difficult, as the disease is rare and early symptoms of babesiosis resemble conditions more likely to be expected in elderly populations, or associated with other more common tick-borne diseases, which might be treated empirically with antibiotics typically prescribed for Lyme disease or anaplasmosis, such as doxycycline. Antibiotics alone are not effective against babesiosis. Furthermore, coinfection of *I. scapularis* with *B. microti* and *B. burgdorferi* is common; half of the ticks (18/36 = 50.0%) positive for *B. microti* in this study were also infected with *B. burgdorferi*, 1 was triple-infected with *A. phagocytophilum, B. burgdorferi, B. microti* and additional *I. scapularis* from Maryland and Virginia have been found concurrently infected with *A. phagocytophilum*, *B. burgdorferi*, *B. microti*, and *B. miyamotoi* (Milholland et al. 2021b, Fleshman et al. 2022). Practitioners need to be alert to concurrent conditions that might manifest. Additionally, in the temperate climate of eastern Maryland, Virginia, and the Delmarva Peninsula, hunters, hikers, gardeners, and outdoor workers are likely to encounter adult *I. scapularis* during their winter period of activity. Although nymphal *I. scapularis* are considered to be the most important vectors to humans because of their small size and warm weather seasonality, adult ticks are also quite tiny and may feed undetected long enough to transmit their myriad pathogens (Casteel and Sonenshine 1996).

The landscape of ticks in the mid-Atlantic area is dynamic, and the status of tick populations and the pathogens they carry will likely continue to change. In Maryland, Virginia, West Virginia, and the District of Columbia, where *I. scapularis* is spreading, and in southeastern Virginia and on the Delmarva Peninsula, where *I. scapularis* is established and *I. keiransi* is advancing, babesiosis is likely emerging, unrecognized and therefore under-reported. Delaware, Maryland, Virginia and West Virginia have been designated by CDC as Lyme disease high-incidence states, having a 3-yr average incidence of greater than 10 cases per 100,000 persons (Kugeler et al. 2024). Babesiosis would be expected to increase in these states because Lyme disease is the harbinger of babesiosis. Public health surveillance, including conducting thorough investigations of all potential human cases of babesiosis and conducting tick surveillance whenever possible, is paramount and should be expanded. Education about this emerging risk, including how to prevent infection in the first place, how to recognize infection, and appropriate treatment, should be increased for medical providers, public health practitioners and the general population.

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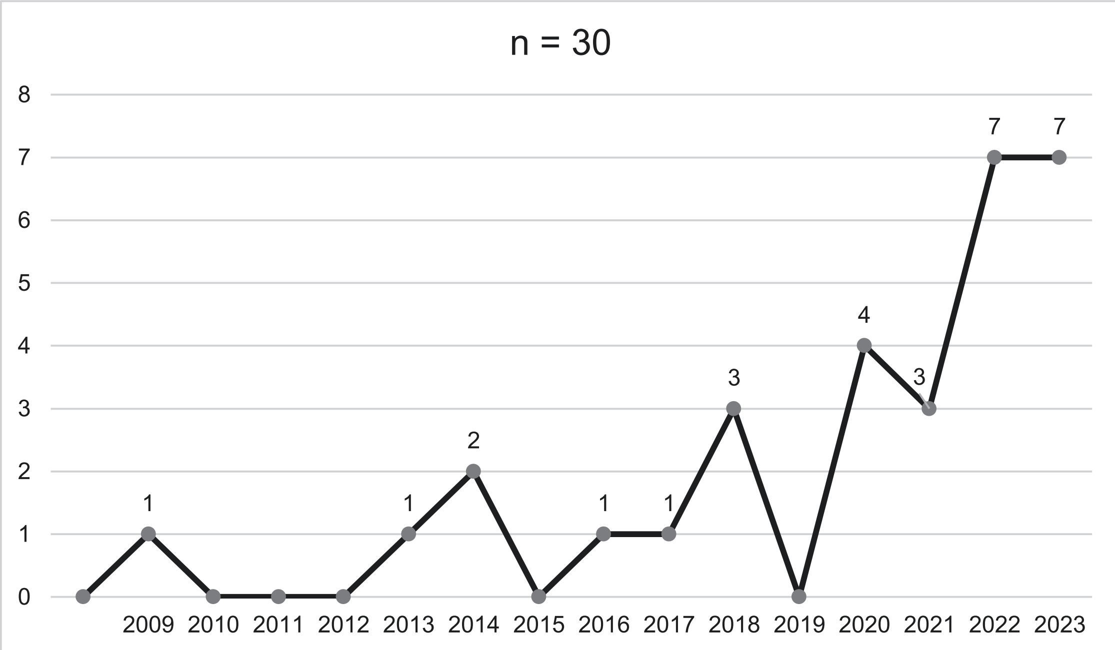

Fig. 2. Number of autochthonous cases of human babesiosis reported in Maryland, District of Columbia, Virginia, West Virginia, 2009 to 2023, *n* = 30 cases.

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Author contributions

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