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Prevalence of single and coinfections of human pathogens in *Ixodes* ticks from five geographical regions in the United States, 2013–2019

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Abstract

As the geographic distributions of medically important ticks and tick-borne pathogens continue to expand in the United States, the burden of tick-borne diseases continues to increase along with a growing risk of coinfections. Coinfection with multiple tick-borne pathogens may amplify severity of disease and complicate diagnosis and treatment. By testing 13,400 *Ixodes* ticks from 17 US states spanning five geographical regions for etiological agents of Lyme disease (*Borrelia burgdorferi* sensu stricto [s.s.] and *Borrelia mayonii*), *Borrelia miyamotoi* disease (*Borrelia miyamotoi*), anaplasmosis (*Anaplasma phagocytophilum*), and babesiosis (*Babesia microti*) we show that *B. burgdorferi* s.s. was the most prevalent and widespread pathogen. *Borrelia miyamotoi*, *A. phagocytophilum*, and *B. microti* were widespread but less prevalent than *B. burgdorferi* s.s. Coinfections with *B. burgdorferi* s.s. and *A. phagocytophilum* or *B. microti* were most common in the Northeast and occurred at rates higher than expected based on rates of single infections in that region.

Keywords

Ticks; *Ixodes* ; *Borrelia* ; *Anaplasma* ; *Babesia* ; Surveillance; Coinfection

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Disclaimers

The findings and conclusions of this study are by the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

CRediT authorship contribution statement

Aine Lehane: Data curation, Methodology, Visualization, Formal analysis, Writing - original draft. **Sarah E. Maes:** Data curation, Methodology, Writing - original draft. **Christine B. Graham:** Data curation, Methodology, Writing - original draft. **Emma Jones:** Formal analysis, Methodology, Visualization, Writing - original draft. **Mark Delorey:** Formal analysis, Methodology. **Rebecca J. Eisen:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing.

1. Introduction

Tick-borne diseases are becoming increasingly more common and geographically widespread in the United States (Rosenberg et al., 2018). This trend is explained, in part, by expanding ranges of medically important ticks and an accelerating rate of new tick-borne pathogen discovery (Eisen and Paddock, 2020). The majority of tick-borne disease cases are associated with the blacklegged tick, *Ixodes scapularis*, a tick that was restricted to focal regions of the U.S. and not even considered a medically important tick before the 1970s but is now ubiquitous in the eastern U.S. and recognized as a vector of seven human pathogens (Eisen and Eisen, 2018; Eisen et al., 2016). As the geographic range of this tick and its associated pathogens continue to expand, the human population at risk for exposure to *I. scapularis*-borne infections increases as does the risk of coinfections. Coinfection with multiple *Ixodes*-borne pathogens may increase severity of disease and complicate diagnosis and treatment (Belongia, 2002; Krause et al., 1996). Understanding the true rate of coinfections in humans is challenging as many epidemiological studies reporting human coinfection fail to distinguish concurrent and sequential infections (Chmielewska-Badora et al., 2012; Mitchell et al., 1996).

Humans can become coinfected from the bite of a single tick that is infected with and transmits multiple pathogens, or by simultaneous or successive bites from multiple ticks each transmitting a different pathogen. Assessing differences in prevalence of single and coinfections in host-seeking ticks across regions and life stages can aid in estimating acarological risk of infections or coinfections in humans. While several previous studies have reported prevalence of single or coinfections in *Ixodes* ticks at local scales (Adelson et al., 2004; Aliota et al., 2014; Hersh et al., 2014; Holden et al., 2003; Holman et al., 2004; Hutchinson et al., 2015; Johnson et al., 2017, 2018; Little and Molaei, 2020; Piesman et al., 1986; Prusinski et al., 2014; Schauber et al., 1998; Schulze et al., 2013, 2005; Schwartz et al., 1997; Varde et al., 1998; Xu et al., 2016), comparison across regions is often complicated by use of varying pathogen detection assays, differences in the suite of pathogens included, and the blood feeding status of the ticks tested. In this study we used a consistent pathogen detection assay (Graham et al., 2018) and we restricted testing to host-seeking nymphs and adults. We tested 13,400 *I. scapularis* and *I. pacificus* ticks collected from 2013 through 2019 from 17 US states spanning five geographical regions for etiological agents of Lyme disease (*Borrelia burgdorferi* sensu stricto [s.s.] and *Borrelia mayonii*), Borrelia miyamotoi disease (*Borrelia miyamotoi*), anaplasmosis (*Anaplasma phagocytophilum*), and babesiosis (*Babesia microti*). We 1) summarize single- and coinfection prevalence for these pathogens in ticks by species, life stage and geographic region, and 2) evaluate if coinfections occur more commonly than expected based on prevalence of single infections.

2. Methods

2.1. Collection sites

From 2013 through 2019, host-seeking *I. scapularis* or *I. pacificus* nymphs or adults were collected by dragging, flagging or CO_2 trapping from a total of 261 counties in 17 states and Washington D.C. (Fig. 1). Sampling was conducted either as part of the Centers for Disease

Control and Prevention's (CDC) national tick and tick-borne pathogen surveillance program (CDC, 2018b; Eisen and Paddock, 2020) or as part of collaborative research projects with academic or public health partners. All ticks were submitted to CDC's Division of Vector-Borne Diseases, Bacterial Diseases Branch for pathogen testing.

2.2. Pathogen detection

To extract DNA, we homogenized individual ticks in lysis buffer using a Mini-Beadbeater-96 (BioSpec Products, Bartlesville, OK, USA) and then processed approximately 40 % of each tick lysate using the QIAcube HT system and the *cador* Pathogen 96 QIAcube HT kit (Qiagen, Valencia, CA, USA) as described previously (Graham et al., 2018; Johnson et al., 2017, 2018), or using the KingFisher Flex and the Mag-Max CORE Nucleic Acid Purification Kit (ThermoFisher Scientific, Waltham, MA). To prepare homogenates for processing on the KingFisher Flex, we followed the manufacturer's "complex method" with modifications. Briefly, we mixed 200 μL homogenate with 450 μL lysis solution for 5 min at moderate speed, then we mixed 30 μL bead/proteinase K mix with the lysate for 2 min at vigorous speed. Finally, we added 350 μL binding solution and processed the samples using the MagMax_CORE_Flex_96W program (ThermoFisher Scientific).

All ticks were screened for *B. burgdorferi* s.s., *B. mayonii*, *B. miyamotoi*, *A. phagocytophilum*, and *B. microti* (Table 1) except for a minority of ticks that were not tested for *B. mayonii* because they were submitted before *B. mayonii* was integrated into the standard testing algorithm in 2017.

First, using probe-based real-time PCR reactions, we screened all samples using a series of paired multiplex assays to detect multiple targets from each pathogen: genes encoding P44 outer membrane surface proteins (p44) and major surface protein 4 (msp4) for *A. phagocytophilum*; genes encoding secreted antigen 1 (sa1) and 18S rRNA (18S) for *B. microti*; a flagellin filament cap gene (fliD) for *B. burgdorferi* s.s. and *B. mayonii*; and a genomic *Borrelia* target (gB31) present in *B. burgdorferi* s.s. and *B. miyamotoi* (Hojgaard et al., 2014) or a 16S rDNA (16S) a pan-*Borrelia* target for *Borrelia* spp. (Graham et al., 2018). Reaction conditions were as described previously (Graham et al., 2018; Hojgaard et al., 2014). The multiplex assays also incorporated an *I. scapularis* actin target that was previously shown to verify DNA integrity in both *I. scapularis* and *I. pacificus* (Graham et al., 2018, 2016).

We screened all *Borrelia*-positive ticks for *B. miyamotoi* using a pair of *B. miyamotoi* specific targets for adenylosuccinate lyase (purB) and glycerophosphodiesterase (glpQ) genes as described previously (Graham et al., 2016). Among the small minority of ticks tested before 2017, we identified *B. burgdorferi* s.s.-positive samples by amplifying and sequencing *B. burgdorferi* s.l. ClpA protease subunit A (*clpA*) and/or Dipeptidyl amino-amino-peptidase (*pepX*) targets from all *B. burgdorferi* s.l.-positive *I. pacificus* and from a representative sample of *B. burgdorferi* s.l.-positive *I. scapularis* as described previously (Johnson et al., 2017). To detect and differentiate *B. burgdorferi* s.s. and *B. mayonii* in all *Borrelia*-positive samples tested after 2017, we used a pair of TaqMan real-time PCR duplex assays targeting the oligopeptide permease periplasmic A2 gene (oppA2) as described

previously (Graham et al., 2018). All PCR reactions were performed using a C1000 Touch thermal cycler with a CFX96 real time system (Bio-Rad, Hercules, CA, USA). We analyzed the samples using the CFX Manager 3.1 software (Bio-Rad) with the quantitation cycle (Cq) determination set to regression.

2.3. Statistical analysis

We calculated the infection prevalence and associated 95 % confidence intervals for all pathogens, and all possible combinations of pathogens for each state and each geographic region. The 95 % confidence intervals were calculated using the Wilson-score method for binomial probabilities. Having computed confidence intervals for single parameters, we use these to compare prevalence among regions, realizing that this increases our Type II error.

Permutation tests were used to determine whether an observed co-infection prevalence was different than the expected coinfection prevalence based on single infections. If coinfections occur independently, then coinfection prevalence equals the product of the marginal infection prevalences. Approximate null distributions of coinfection prevalences (assumes independence of infections) were constructed by permuting testing results for one of the pathogens ten thousand times to determine the prevalence of coinfection. The observed coinfection prevalence was then compared to the 2.5th and 97.5th quantiles of the null distribution to assess whether the observed coinfection prevalence fell within this boundary. Observed coinfection prevalences that fell outside of this boundary were assumed to occur either more or less than expected than if infections occur independently. All analyses were conducted in R (Team, 2013).

3. Results

Of the 13,400 *Ixodes* ticks tested from 17 U.S. states and the District of Columbia, 6,059 (45.21 %) were adults and 7,341 (54.78 %) were nymphs (Fig. 1). Host seeking nymphs were rarely submitted from the southeastern U.S., where adults were the predominant life stage submitted for testing. In general, with the exception of *B. burgdorferi* s.s. in the Northwest, infection prevalence was higher in adults compared with nymphs (Table 1).

Borrelia burgdorferi s.s. was the most prevalent and geographically widespread pathogen, detected in each of the states from which ticks were submitted except for Kentucky, Alabama, Mississippi, and Oregon; however, sample sizes were relatively low from most of these states. Among all ticks tested, 18.20 % (17.33–19.10 %) of nymphs and 37.67 % (36.44–38.90 %) of adults were infected with *B. burgdorferi* s.s. (Table 1). Infection prevalence in nymphs (21.26 % [19.11–23.58 %]) and adults (58.04 % [55.94–60.11 %]) was highest in the Northeast compared with all other regions. Prevalence of *B. burgdorferi* s.s. was similar between the Mid-Atlantic (16.85 % [15.31–18.52 %]; 39.95 % [35.37–44.72 %], in nymphs and adults, respectively) and Midwest (17.99 % [16.82–19.22%]; 33.14 % [31.30–35.04 %]). Nymphal infection prevalence was significantly lower in the Southeast (0.00 % [0.00–0.39 %]) compared with all other regions, whereas prevalence of infection in adults was similar between the Southeast (1.65 % [0.71–3.80 %]) and Northwest (2.28 % [1.43–3.63 %]) and lower in both these regions compared with all others.

Borrelia miyamotoi, *A. phagocytophilum*, and *B. microti* were widespread but less prevalent than *B. burgdorferi* s.s. (Table 1). *Borrelia miyamotoi* was detected in ticks collected from each region, with nymphal infection prevalence similar among the Northeast (1.20 % [0.71–1.92 %), Midwest (1.17 % [0.88–1.55 %]) and Mid-Atlantic (1.29 % [0.89–1.87 %]), which trended higher than nymphal infection prevalence in the Northwest and the Southeast where infections were not detected in tested nymphs; prevalence of infection in adult ticks was similar among regions with an overall average of 1.37 % (1.10–1.70 %) infected. *Anaplasma phagocytophilum* was detected in ticks from each region except the Southeast, with highest prevalence of infection recorded in the Northeast (5.76 % [4.62–7.17 %] and 8.07 % [6.99–9.30 %] in nymphs and adults, respectively). In the Northeast, *B. microti* was detected at similar prevalence (5.69 % [4.55–7.10 %] and 3.53 % [2.83–4.39 %] in nymphs and adults, respectively) to *A. phagocytophilum*. *Babesia microti* was less commonly detected in the Midwest (2.46 % [2.02–2.99 %] in nymphs; 0.29 % [0.14–0.59 %] in adults) compared with the Northeast and within the Midwest, *B. microti* was less prevalent in ticks compared with *A. phagocytophilum* (4.03 % [3.46–4.69 %] in nymphs and 3.53 % [2.86–4.33 %] in adults). *Babesia microti* was detected in only a single state (Virginia) in the Mid-Atlantic region and overall prevalence for that region was low (0.09 % [0.02–0.35 %] in nymphs and 0.00 % [0.00–0.91 % in adults); no *B. microti* infections were detected in the Southeast or Northwest. *Borrelia mayonii* was detected only in nymphal ticks from Wisconsin (0.32 % [0.11–0.94 %]) and Minnesota (0.60 % [0.34–1.04 %]) and occurred at very low prevalence (<1 %) (Table 1).

Coinfections were more common in the Northeast compared with other regions (Table 2). Looking only at the three most common pathogens (*B. burgdorferi* s.s., *A. phagocytophilum* and *B. microti*), coinfections were most commonly detected in the Northeast where *B. burgdorferi* s.s. and either *A. phagoctyphilum* or *B. microti* were reported in roughly 3% of nymphs; approximately 1% of nymphs were coinfected with *A. phagocytophilum* and *B. microti*. Compared with the Northeast, coinfection rates were substantially lower in the Midwest and Mid-Atlantic and no coinfections were detected in ticks tested from the Southeast or Northwest (Table 2).

Coinfections with *B. burgdorferi* s.s. and either *A. phagocytophilum* or *B. microti* were observed more frequently than expected based on prevalence of single infections in the Northeast and Midwest, but this trend was not consistent in the Mid-Atlantic or Northwest where coinfections occurred at rates expected or lower than expected based on prevalence of single infections (Fig. 2).

4. Discussion

Surveillance of host-seeking ticks and pathogens in these ticks provide data that are complementary to human disease surveillance, which typically report human disease cases based on state or county of residence, rather than location of exposure. Such reporting of human cases may be misconstrued to give the false impression that risk of exposure to tick-borne infections is more geographically widespread than is real. Because of their limited mobility, testing host-seeking ticks provides spatially precise estimates of local infection presence and prevalence (Eisen and Paddock, 2020). Improved understanding of

where in the United States ticks are biting people and which pathogens they carry can aid in resolving where exposure to tick-borne disease agents occurs. Such information is useful for targeting the delivery of prevention strategies to communities at risk for *Ixodes*-associated diseases. Moreover, tick surveillance data can provide estimates of human risk of exposure to tick-borne pathogens that cause diseases that are not nationally notifiable and for which information on the distribution of human disease cases therefore is limited (e.g., B. miyamotoi disease) (Eisen and Paddock, 2020).

Among the thousands of *Ixodes* ticks we tested for five human pathogens, *B. burgdorferi* s.s. was overwhelmingly the most common and was detected in each of the five geographical regions with an overall prevalence of 18 % in nymphs and 38 % in adults. By contrast, *B. mayonii*, which also causes Lyme disease, was the most geographically restricted and the least commonly detected pathogen, found only in the Midwest and in less than 1 % of ticks from two states in that region. Regional trends in the prevalence of *B. burgdorferi* s.s. infection in ticks are consistent with epidemiological trends showing greatest risk of Lyme disease concentrated in the Northeast, Mid-Atlantic and upper Midwest where host-seeking infected nymphs are more commonly encountered than in other regions of the United States (CDC, 2018a; Diuk-Wasser et al., 2012). Notably, prevalence of *B. burgdorferi* s.s. is relatively lower in areas where ticks feed commonly on lizards that are refractory to infection (e.g, the Southeast and West compared with the Northeast, Mid-Atlantic and upper Midwest); extensive feeding of *I. pacificus* nymphs on lizards that are capable of clearing *B. burgdorferi* s.s. from feeding ticks also contributes to explaining the observed lower prevalence of infection in adults compared with nymphs in the west (Lane and Quistad, 1998; Eisen et al., 2004a,b). Although vector ticks are widely distributed throughout the eastern and Pacific Coast states (Eisen et al., 2016), we report a more limited distribution of Lyme disease spiro-chetes, consistent with previous studies showing that *B. burgorferi* s.s. is rare in host-seeking *I. scapularis* nymphs from the southeast (Diuk--Wasser et al., 2012; Stromdahl and Hickling, 2012). Owing to their small size, which allows them to go undetected while feeding long enough for transmission to occur, nymphs are believed to contribute more than adults to the burden of Lyme disease (Eisen, 2018). However, in the southeastern U.S. where nymphs rarely ascend vegetation, adults might more commonly make contact with humans and cause human infections (Hickling et al., 2018; Stromdahl and Hickling, 2012). Among the small numbers of nymphs submitted from the southeast, we failed to detect *B. burgdorferi* s.s. in any; infections were detected in adult ticks, but at significantly lower prevalence than in other eastern regions. Limited contact between humans and infected nymphs, coupled with low prevalence of *B. burgdorferi* s.s. infection in adult ticks which are more likely than nymphs to be detected and removed prior to transmission occurring, contributes to explain why Lyme disease infections are less common in the Southeast compared with the Northeast, Mid-Atlantic and Midwest.

Similarly, human anaplasmosis and babesiosis cases are reported most commonly from the Northeast where prevalence of infection in the ticks was higher than for other regions (CDC, 2018a). Although consistent with reported disease trends, acarological risk of exposure to *A. phagocytophilum* might be over-estimated in our study because the pathogen detection assay employed does not distinguish the rodent-associated *A. phagocytophilum* variant (*A. phagocytophilum*-ha), which causes human infection, from the deer-associated

variant (*A. phagocytophilum*-variant 1), which does not cause human disease (Graham et al., 2018). Borrelia miyamotoi disease is not a nationally notifiable condition, but consistent with other studies, our data suggest potential risk for exposure to infected ticks is geographically widespread, but the likelihood of encountering an infected tick is generally low (Wagemakers et al., 2015).

Incidence of coinfections in humans is not monitored through national surveillance systems. Our data suggest that risk of coinfections with *Ixodes*-borne pathogens is greatest in the Northeast where prevalence of the three most common pathogens (*B. burgdorferi* s.s., *A. phagocytophilum*, and *B. microti*) was highest and the prevalence of coinfections in ticks was higher than expected based on frequency of single infections. We report prevalence of coinfections similar to studies reviewed recently that showed coinfection prevalence in *I. scapularis* ranging from 1 to 28%, but commonly with fewer than 5–10 % of ticks coinfected (Eisen and Eisen, 2018). Previous studies suggested that *B. burgdorferi* s.s. and *Ba. microti* co-occur in *I. scapularis* more frequently than expected based on frequencies of individual infections, and this was explained by a shared reservoir host and because *B. burgdorferi* s.s. infection may facilitate *Ba. microti* transmission (Diuk-Wasser et al., 2016; Eisen and Eisen, 2018). Here we showed higher than expected rates of coinfection in the Northeast and in nymphs from the Midwest, but coinfection prevalence was observed at rates expected or lower than expected in other regions (Fig. 2). This might be explained by differences in host communities among regions, or attributable to the relatively low rates of *B. microti* outside the Northeast and Midwest where the pathogen has more recently established.

Although our data, derived using a common testing algorithm, provide insights into acarological risk of exposure to five *Ixodes*-associated pathogens and the findings are generally consistent with epidemiological trends, sampling was not conducted systematically. Thus, we caution against extrapolating results across regions to states that were not included in this assessment. Notably, several states that historically reported a high incidence of Lyme disease in the eastern U.S. (e.g., Pennsylvania, New Jersey, Rhode Island, Connecticut, Massachusetts and most counties in New York) and California in the western U.S. where incidence of *Ixodes pacificus*-associated diseases is generally higher than other western states included here (CDC, 2018a), were not represented in our study. Moreover, several southern states that typically report low incidence of *Ixodes*-associated diseases and low prevalence of infection in ticks, were not included (Diuk-Wasser et al., 2012; Stromdahl and Hickling, 2012). The reason for this is, in part, because recent tick surveillance efforts for which CDC provided testing support were differentially targeted to “leading edge” states or those neighboring states reporting high incidence of Lyme disease (Schwartz et al., 2017). Although tick surveillance was conducted in some high incidence states, several conduct their own tick testing and therefore pathogen data from these states were not included in our testing database. In addition, prevalence of infection in ticks described at the state level should not be assumed to be consistently observed among localities within the state. Indeed, previous studies have noted significant variability in infection prevalence among sampling sites (Johnson et al., 2017; Prusinski et al., 2014).

The data presented here report coarse trends in acarological risk of exposure to five *Ixodes*-borne infections across the U.S. Owing to lack of sufficient data, we did not explicitly

present variability in infection prevalence among sampling sites within states or among years, which can be considerable. Nonetheless, we described regional trends that might be explained by multiple influences including, but not limited to: spatial variability in host abundance and composition, host-seeking phenology of ticks, and length of time pathogens have been established in a region (Lane et al., 1991; LoGiudice et al., 2003; Gatewood et al., 2009; Stromdahl et al., 2014). Continuing national tick surveillance efforts should provide improved information by providing estimates of the distribution and abundance of host-seeking ticks and presence and prevalence of human pathogens within ticks with greater coverage than presented here. Documentation of the expanding distribution of ticks and tick-borne pathogens serves as an important reminder of the urgent need to improve strategies to prevent human-tick encounters and ultimately reduce the burden of tick-borne diseases in the U.S.

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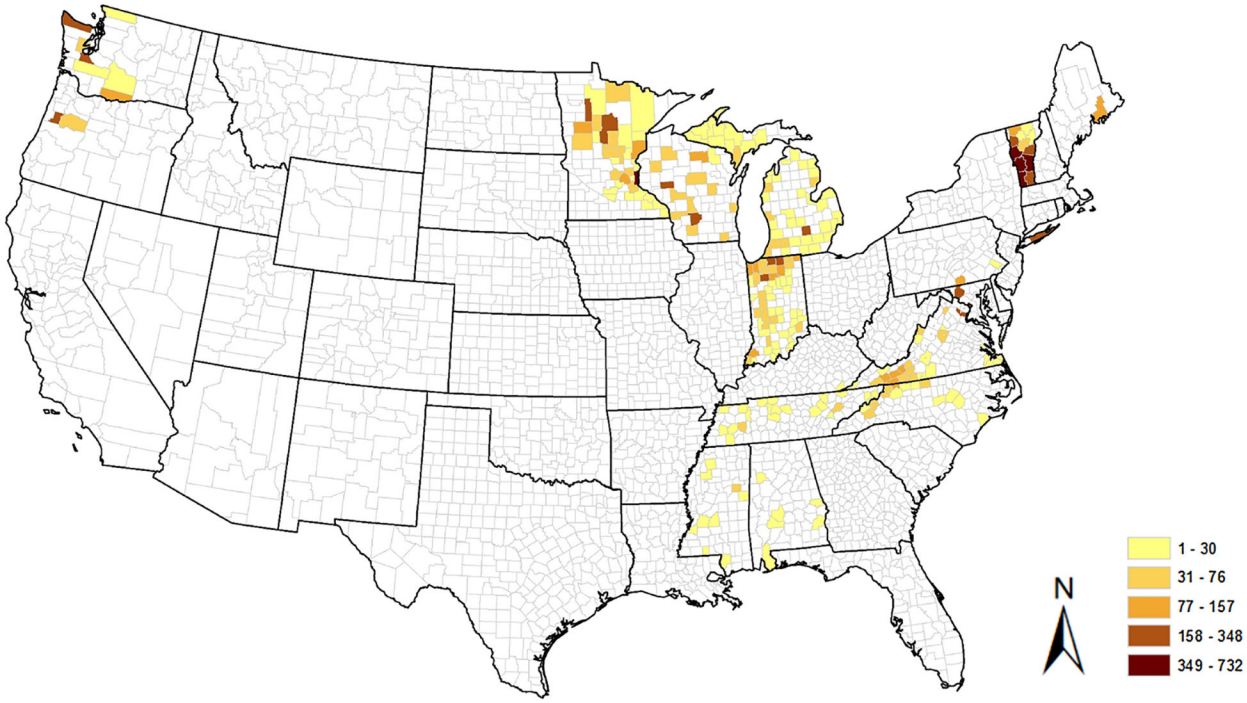

Fig. 1.
Number of *Ixodes* ticks tested for presence of pathogens per county.

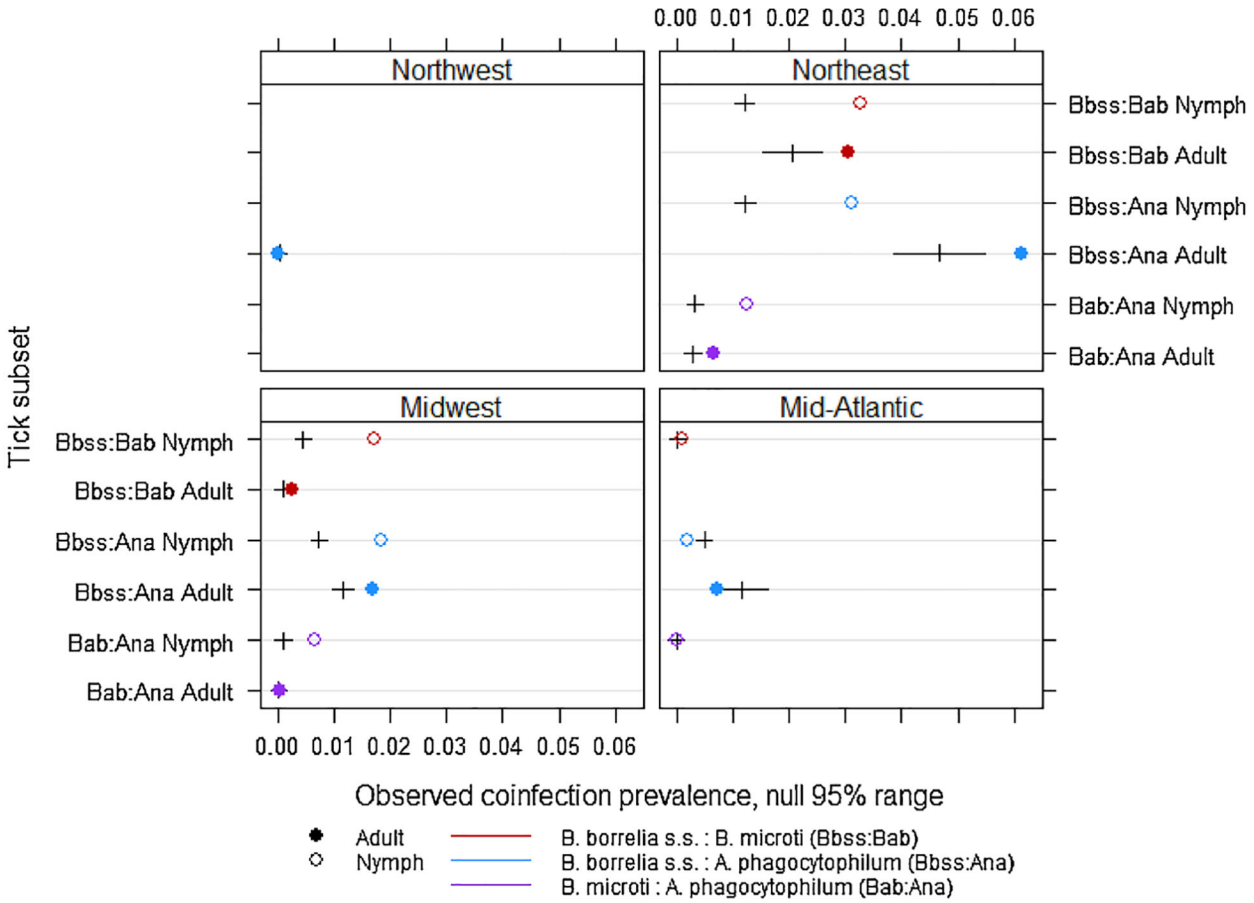

Fig. 2.

Observed coinfection prevalence and the null 95 % range estimated with permutation tests by tick life stage and geographical region. Ticks sampled from the Southeastern region did not have enough coinfections to be included in the permutation analysis.

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Table 1

Prevalence of human pathogens by *Ixodes* species and life-stage by state and region, 2013 – 2019.

Region State[†]	Species, life stage	Total no. positive ticks (% positive [95 % C.I.])									
			***Borrelia burgdorferi* s.s.**		***Borrelia miyamotoi***		***Borrelia mayonii*** [††]		***Anaplasma phagocytophilum***		***Babesia microti***
Northeast											
ME	*I. scapularis*	No. ticks tested		No. ticks tested		No. ticks tested		No. ticks tested		No. ticks tested	
	Nymph	154	27 (17.53 [12.34–24.31])	154	1 (0.65 [0.03–3.59])	–	–	154	5 (3.25 [1.39–7.37])	154	6 (3.90 [1.80–8.24])
	Adult	–	–	–	–	–	–	–	–	–	–
NY	*I. scapularis*										
	Nymph	299	50 (16.72 [12.92–21.37])	299	9 (3.01 [1.59–5.62])	–	–	299	27 (9.03 [6.28–12.82])	299	36 (12.04 [8.82–16.22])
	Adult	–	–	–	–	–	–	–	–	–	–
PA	*I. scapularis*										
	Nymph	115	26 (22.61 [15.92–31.07])	115	0 (0.00 [0.00–3.23])	1	0 (0.00 [0.00–94.87])	115	3 (2.61 [0.89–7.39])	115	0 (0.00 [0.00–3.23])
	Adult	–	–	–	–	–	–	–	–	–	–
VT	*I. scapularis*										
	Nymph	716	170 (23.74 [20.77–26.99])	716	5 (0.70 [0.30–1.62])	716	0 (0.00 [0.00–0.53])	716	39 (5.45 [4.01–7.36])	716	31 (4.33 [3.07–6.08])
	Adult	2152	1249 (58.04 [55.94–60.11])	2155	24 (1.11 [0.75–1.65])	2153	0 (0.00 [0.00–0.18])	2155	174 (8.07 [7.00–9.30])	2155	76 (3.53 [2.83–4.39])
Total											
	Nymph	1284	273 (21.26 [19.11–23.58])	1284	15 (1.20 [0.71–1.92])	717	0 (0.00 [0.00–0.53])	1284	74 (5.76 [4.62–7.17])	1284	73 (5.69 [4.55–7.10])
	Adult	2152	1249 (58.04 [55.94–60.11])	2155	24 (1.11 [0.75–1.65])	2153	0 (0.00 [0.00–0.18])	2155	174 (8.07 [6.99–9.30])	2155	76 (3.53 [2.83–4.39])
Mid-Atlantic											
DC	*I. scapularis*										
	Nymph	253	62 (24.51 [19.61–30.16])	253	2 (0.79 [0.22–2.84])	–	–	253	1 (0.40 [0.02–2.20])	253	0 (0.00 [0.00–1.50])
	Adult	–	–	–	–	–	–	–	–	–	–

Region State†	Species, life stage	Total no. positive ticks (% positive [95 % C.I.])									
			Borrelia burgdorferi s.s.		*Borrelia miyamotoi*		*Borrelia mayonii* ††		*Anaplasma phagocytophilum*		*Babesia microti*
KY	*I. scapularis*										
	Nymph	13	0 (0.00 [0.00–22.81])	13	0 (0.00 [0.00–22.81])	13	0 (0.00 [0.00–22.81])	13	0 (0.00 [0.00–22.81])	13	0 (0.00 [0.00–22.81])
	Adult	–	–	–	–	–	–	–	–	–	–
MD	*I. scapularis*										
	Nymph	168	39 (23.21 [17.47–30.15])	168	4 (2.38 [0.93–5.96])	–	–	168	4 (2.38 [0.93–5.96])	168	0 (0.00 [0.00–2.24])
	Adult	–	–	–	–	–	–	–	–	–	–
NC	*I. scapularis*										
	Nymph	378	46 (12.17 [9.25–15.85])	378	4 (1.06 [0.41–2.69])	378	0 (0.00 [0.00–1.01])	378	5 (1.32 [0.57–3.06])	378	0 (0.00 [0.00–1.01])
	Adult	89	38 (42.70 [32.93–53.06])	89	1 (1.12 [0.06–6.09])	89	0 (0.00 [0.00–4.14])	89	2 (2.25 [0.62–7.83])	89	0 (0.00 [0.00–4.14])
VA	*I. scapularis*										
	Nymph	1276	205 (16.07 [14.15–18.18])	1277	17 (1.33 [0.83–2.12])	804	0 (0.00 [0.00–0.48])	1277	51 (3.99 [3.05–5.21])	1277	2 (0.16 [0.04–0.57])
	Adult	329	129 (39.21 [34.09–44.58])	329	9 (2.74 [1.45–5.12])	329	0 (0.00 [0.00–1.15])	329	10 (3.04 [1.66–5.50])	329	0 (0.00 [0.00–1.15])
Total											
	Nymph	2088	352 (16.85 [15.31–18.52])	2089	27 (1.29 [0.89–1.87])	1195	0 (0.00 [0.00–0.32])	2089	61 (2.92 [2.28–3.73])	2089	2 (0.09 [0.02–0.35])
	Adult	418	167 (39.95 [35.37–44.72])	418	10 (2.39 [1.30–4.35])	418	0 (0.00 [0.00–0.91])	418	12 (2.87 [1.65–4.95])	418	0 (0.00 [0.00–0.91])
Midwest											
IN	*I. scapularis*										
	Nymph	721	107 (14.84 [12.43–17.62])	721	10 (1.39 [0.76–2.53])	721	0 (0.00 [0.00–0.53])	721	6 (0.83 [0.38–1.80])	721	0 (0.00 [0.00–0.53])
	Adult	1686	612 (36.30 [34.04–38.62])	1686	21 (1.25 [0.82–1.90])	1686	0 (0.00 [0.00–0.23])	1686	41 (2.43 [1.80–3.28])	1686	0 (0.00 [0.00–0.23])
MI	*I. scapularis*										
	Nymph	287	16 (5.57 [3.46–8.86])	287	1 (0.35 [0.02–1.95])	287	0 (0.00 [0.00–1.32])	287	6 (2.09 [0.96–4.49])	287	0 (0.00 [0.00–1.32])
	Adult	535	113 (21.12 [17.87–24.78])	535	4 (0.75 [0.29–1.91])	535	0 (0.00 [0.00–0.71])	536	31 (5.78 [4.10–8.09])	536	0 (0.00 [0.00–0.71])

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Region State†	Species, life stage	Total no. positive ticks (% positive [95 % C.I.])									
			***Borrelia burgdorferi* s.s.**		***Borrelia miyamotoi***		***Borrelia mayonii* ††**		***Anaplasma phagocytophilum***		***Babesia microti***
MN	*I. scapularis*										
	Nymph	2004	464 (23.15 [21.36–25.05])	2004	19 (0.95 [0.61–1.48])	2004	12 (0.60 [0.34–1.04])	2004	109 (5.44 [4.53–6.52])	2004	85 (4.24 [3.44–5.21])
	Adult	148	48 (32.43 [25.42–40.34])	148	4 (2.70 [1.06–6.74])	148	0 (0.00 [0.00–2.53])	148	7 (4.73 [2.31–9.44])	148	0 (0.00 [0.00–2.53])
WI	*I. scapularis*										
	Nymph	929		930		930		930		930	
			122 (13.13 [11.11–15.46])		16 (1.72 [1.06–2.78])		3 (0.32 [0.11–0.94])		38 (4.09 [2.99–5.56])		12 (1.29 [0.74–2.24])
	Adult	69	35 (50.72 [39.21–62.17])	69	4 (5.80 [2.28–13.98])	69	0 (0.00 [0.00–5.27])	69	7 (10.14 [5.00–19.49])	69	7 (10.14 [5.00–19.49])
Total											
	Nymph	3941	709 (17.99 [16.82–19.22])	3942	46 (1.17 [0.88–1.55])	3942	15 (0.38 [0.23–0.62])	3942	159 (4.03 [3.46–4.69])	3942	97 (2.46 [2.02–2.99])
	Adult	2438	808 (33.14 [31.30I 35.04])	2438	33 (1.35 [0.97–1.89])	2438	0 (0.00 [0.00–0.16])	2439	86 (3.53 [2.86–4.33])	2439	7 (0.29 [0.14I 0.59])
Southeast											
AL	*I. scapularis*	n		n		n		n		n	
	Nymph	3	0 (0.00 [0.00–56.15])	3	0 (0.00 [0.00–56.15])	3	0 (0.00 [0.00–56.15])	3	0 (0.00 [0.00–56.15])	3	0 (0.00 [0.00–56.15])
	Adult	22	0 (0.00 [0.00–14.87])	22	0 (0.00 [0.00–14.87])	22	0 (0.00 [0.00–14.87])	22	0 (0.00 [0.00–14.87])	22	0 (0.00 [0.00–14.87])
MS	*I. scapularis*										
	Nymph	–	–	–	–	–	–	–	–	–	–
	Adult	70	0 (0.00 [0.00–5.20])	70	0 (0.00 [0.00–5.20])	70	0 (0.00 [0.00–5.20])	70	0 (0.00 [0.00–5.20])	70	0 (0.00 [0.00–5.20])
TN	*I. scapularis*										
	Nymph	3	0 (0.00 [0.00–56.15])	3	0 (0.00 [0.00–56.15])	3	0 (0.00 [0.00–56.15])	3	0 (0.00 [0.00–56.15])	3	0 (0.00 [0.00–56.15])
	Adult	211	5 (2.37 [1.02–5.43])	211	2 (0.95 [0.26–3.39])	211	0 (0.00 [0.00–1.79])	211	0 (0.00 [0.00–1.79])	211	0 (0.00 [0.00–1.79])
Total											
	Nymph	6	0 (0.00 [0.00–0.39])	6	0 (0.00 [0.00–0.39])	6	0 (0.00 [0.00–0.39])	6	0 (0.00 [0.00–0.39])	6	0 (0.00 [0.00–0.39])

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Region State†	Species, life stage	Total no. positive ticks (% positive [95 % C.I.])									
			***Borrelia burgdorferi* s.s.**		***Borrelia miyamotoi***		***Borrelia mayonii* ††**		***Anaplasma phagocytophilum***		***Babesia microti***
	Adult	303	5 (1.65 [0.71–3.80])	303	2 (0.66 [0.18–2.37])	303	0 (0.00 [0.00–1.25])	303	0 (0.00 [0.00–1.25])	303	0 (0.00 [0.00–1.25])
Northwest											
OR	*I. pacificus*										
	Nymph	–	–	–	–	–	–	–	–	–	–
	Adult	243	0 (0.00 [0.00–1.56])	243	2 (0.82 [0.23–2.95])	243	0 (0.00 [0.00–1.56])	243	0 (0.00 [0.00–1.56])	243	0 (0.00 [0.00–1.56])
WA	*I. pacificus*										
	Nymph	20	1 (5.00 [0.26–23.61])	20	0 (0.00 [0.00–16.11])	15	0 (0.00 [0.00–20.39])	20	0 (0.00 [0.00–16.11])	20	0 (0.00 [0.00–16.11])
	Adult	501	17 (3.39 [2.13–5.37])	501	11 (2.20 [1.23–3.89])	387	0 (0.00 [0.00 – 0.98])	501	8 (1.60 [0.81–3.12])	501	0 (0.00 [0.00 – 0.76])
Total											
	Nymph	20	1 (5.00 [0.89–23.61])	20	0 (0.00 [0.00–16.11])	15	0 (0.00 [0.00–20.39])	20	0 (0.00 [0.00–16.11])	20	0 (0.00 [0.00–16.11])
	Adult	744	17 (2.28 [1.43–3.63])	744	13 (1.75 [1.03–2.97])	630	0 (0.00 [0.00 – 0.61])	744	8 (1.08 [0.55–2.11])	744	0 (0.00 [0.00–0.51])
Total	*Ixodes* spp.										
	Nymph	7336	1335 (18.20 [17.33–19.10])	7341	88 (1.20 [0.97–1.47])	5872	15 (0.26 [0.15–0.42])	7341	294 (4.00 [3.58–4.45])	7341	172 (2.34 [2.02–2.71])
	Adult	5963	2246 (37.67 [36.44–38.90])	5988	82 (1.37 [1.10–1.70])	5850	0 (0.00 [0.00–0.07])	6059	280 (4.62 [4.12–5.18])	6059	83 (1.37 [1.11–1.69])

†ME: Maine; NY: New York; PA: Pennsylvania; VT: Vermont; DC: Washington, D.C.; KY: Kentucky; MD: Maryland; NC: North Carolina; VA: Virginia; IN: Indiana; MI: Michigan; MN: Minnesota; WI: Wisconsin; AL: Alabama; MS: Mississippi; TN: Tennessee; OR: Oregon; WA: Washington.

††Testing for *B. mayonii* was not initiated until 2017, thus samples tested prior to 2017 were not tested for *B. mayonii*.

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Table 2

Prevalence of *Borrelia burgdorferi* s.s., *Anaplasma phagocytophilum*, and *Babesia microti* coinfections by *Ixodes* species and life-stage at the state-level, 2013 – 2019.

Region State[†]	Tick species and life stage	No. ticks tested	Total no. ticks co-infected (% [95 % C.I.])		
			Borrelia burgdorferi* s.s. and *Anaplasma phagocytophilum	***Borrelia burgdorferi* s.s. and *Babesia microti***	***Anaplasma phagocytophilum* and *Babesia microti***
Northeast					
ME	*I. scapularis*				
	Nymph	154	4 (2.6 [1.01–6.49])	3 (1.95 [0.66–5.57])	0 (0.00 [0.00–2.43])
	Adult	–			
NY	*I. scapularis*				
	Nymph	299	9 (3.01 [1.59–5.62])	16 (5.35 [3.32–8.51])	5 (1.67 [0.72–3.85])
	Adult	–			
PA	*I. scapularis*				
	Nymph	115	1 (0.87 [0.04–4.76])	0 (0.00 [0.00–3.23])	0 (0.00 [0.00–3.23])
	Adult	–			
VT	*I. scapularis*				
	Nymph	716	26 (3.63 [2.49–5.27])	23 (3.21 [2.15–4.77])	11 (1.54 [0.86–2.73])
	Adult	2155	132 (6.13 [5.19–.22])	66 (3.06 [2.41–3.88])	14 (0.65 [0.39–1.09])
Total					
	Nymph	1284	40 (3.12 [2.30–4.21])	42 (3.27 [2.42–4.39])	16 (1.25 [0.77–2.01])
	Adult	2155	132 (6.13 [5.19–7.22])	66 (3.06 [2.41–3.88])	14 (0.65 [0.39–1.09])
Mid-Atlantic					
DC	*I. scapularis*				
	Nymph	253	0 (0.00 [0.00–1.50])	0 (0.00 [0.00–1.50])	0 (0.00 [0.00–1.50])
	Adult	–			
KY	*I. scapularis*				
	Nymph	13	0 (0.00 [0.00–22.81])	0 (0.00 [0.00–22.81])	0 (0.00 [0.00–22.81])
	Adult	–			
MD	*I. scapularis*				
	Nymph	168	0 (0.00 [0.00–2.24])	0 (0.00 [0.00–2.24])	0 (0.00 [0.00–2.24])

Region State†	Tick species and life stage	No. ticks tested	Total no. ticks co-infected (% [95 % C.I.])		
			Borrelia burgdorferi* s.s. and *Anaplasma phagocytophilum	***Borrelia burgdorferi* s.s. and *Babesia microti***	***Anaplasma phagocytophilum* and *Babesia microti***
	Adult	–			
NC	*I. scapularis*				
	Nymph	378	0 (0.00 [0.00–1.01])	0 (0.00 [0.00–1.01])	0 (0.00 [0.00–1.01])
	Adult	89	1 (1.12 (0.06–6.09])	0 (0.00 [0.00–4.14])	0 (0.00 [0.00–4.14])
VA	*I. scapularis*				
	Nymph	1277	4 (0.31 [0.12–0.80])	2 (0.16 [0.04–0.57])	0 (0.00 [0.00–0.30])
	Adult	329	2 (0.61 [0.17–2.19])	0 (0.00 [0.00–1.15])	0 (0.00 [0.00–1.15])
Total					
	Nymph	2089	4 (0.19 [0.07–0.49])	2 (0.10 [0.03–0.35])	0 (0.00 [0.00–0.18])
	Adult	418	3 (0.72 [0.24–2.09])	0 (0.00 [0.00–0.91])	0 (0.00 [0.00–0.91])
Midwest					
IN	*I. scapularis*				
	Nymph	721	0 (0.00 [0.00–0.53])	0 (0.00 [0.00–0.53])	0 (0.00 [0.00–0.53])
	Adult	1686	19 (1.13 [0.72–1.75])	0 (0.00 [0.00–0.23])	0 (0.00 [0.00–0.23])
MI	*I. scapularis*				
	Nymph	287	1 (0.35 [0.02–1.95])	0 (0.00 [0.00–1.32])	0 (0.00 [0.00–1.32])
	Adult	536	15 (2.80 [1.70–4.57])	0 (0.00 [0.00–0.71])	0 (0.00 [0.00–0.71])
MN	*I. scapularis*				
	Nymph	2006	63 (3.14 [2.46–4.00])	60 (2.99 [2.33–3.83])	25 (1.25 [0.85–1.83])
	Adult	148	3 (2.03 [0.69–5.79])	0 (0.00 [0.00–2.53])	0 (0.00 [0.00–2.53])
WI	*I. scapularis*				
	Nymph	930	9 (0.97 [0.51–1.83])	8 (0.86 [0.44–1.69])	1 (0.11 [0.01–0.61])
	Adult	69	4 (5.80 [2.28–13.98])	6 (8.70 [4.05–17.70])	1 (1.45 [0.07–7.76])
Total					
	Nymph	3944	73 (1.85 [1.47–2.32])	68 (1.72 [1.36–2.18])	26 (0.66 [0.45–0.96])
	Adult	2439	41 (1.68 [1.24–2.27])	6 (0.25 [0.11–0.54])	1 (0.04 [0.01–0.23])
Southeast					
AL	*I. scapularis*				
	Nymph	3	0 (0.00 [0.00–56.15])	0 (0.00 [0.00–56.15])	0 (0.00 [0.00–56.15])

Region State[†]	Tick species and life stage	No. ticks tested	Total no. ticks co-infected (% [95 % C.I.])		
			Borrelia burgdorferi* s.s. and *Anaplasma phagocytophilum	***Borrelia burgdorferi* s.s. and *Babesia microti***	***Anaplasma phagocytophilum* and *Babesia microti***
	Adult	22	0 (0.00 [0.00–14.87])	0 (0.00 [0.00–14.87])	0 (0.00 [0.00–14.87])
MS	*I. scapularis*				
	Nymph	–			
	Adult	70	0 (0.00 [0.00–5.20])	0 (0.00 [0.00–5.20])	0 (0.00 [0.00–5.20])
TN	*I. scapularis*				
	Nymph	3	0 (0.00 [0.00–56.15])	0 (0.00 [0.00–56.15])	0 (0.00 [0.00–56.15])
	Adult	211	0 (0.00 [0.00–1.79])	0 (0.00 [0.00–1.79])	0 (0.00 [0.00–1.79])
Total					
	Nymph	6	0 (0.00 [0.00–39.03])	0 (0.00 [0.00–39.03])	0 (0.00 [0.00–39.03])
	Adult	303	0 (0.00 [0.00–1.25])	0 (0.00 [0.00–1.25])	0 (0.00 [0.00–1.25])
Northwest					
OR	*I. pacificus*				
	Nymph	–			
	Adult	243	0 (0.00 [0.00–1.56])	0 (0.00 [0.00–1.56])	0 (0.00 [0.00–1.56])
WA	*I. pacificus*				
	Nymph	20	0 (0.00 [0.00–16.11])	0 (0.00 [0.00–16.11])	0 (0.00 [0.00–16.11])
	Adult	501	0 (0.00 [0.00–0.76])	0 (0.00 [0.00–0.76])	0 (0.00 [0.00–0.76])
Total					
	Nymph	20	0 (0.00 [0.00–16.11])	0 (0.00 [0.00–16.11])	0 (0.00 [0.00–16.11])
	Adult	744	0 (0.00 [0.00–0.51])	0 (0.00 [0.00–0.51])	0 (0.00 [0.00–0.51])
Total	*Ixodes* spp.	7343	117 (1.59 [1.33–1.91])	112 (1.53 [1.27–1.83])	42 (0.57 [0.42–0.77])
	Nymph	7343	117 (1.59 [1.33–1.91])	112 (1.53 [1.27–1.83])	42 (0.57 [0.42–0.77])
	Adult	6059	176 (2.90 [2.51–3.36])	72 (1.19 [0.94–1.49])	15 (0.25 [0.15I 0.41])

[†]ME: Maine; NY: New York; PA: Pennsylvania; VT: Vermont; DC: Washington, D.C.; KY: Kentucky; MD: Maryland; NC: North Carolina; VA: Virginia; IN: Indiana; MI: Michigan; MN: Minnesota; WI: Wisconsin; AL: Alabama; MS: Mississippi; TN: Tennessee; OR: Oregon; WA: Washington.