Borrelia miyamotoi in host-seeking *Ixodes ricinus* ticks in England

K. M. HANSFORD[1]*, M. FONVILLE[2], S. JAHFARI[2], H. SPRONG[2]
AND J. M. MEDLOCK[1]

[1] *Medical Entomology & Zoonoses Ecology Group, MRA&BS, Emergency Response Department, Public Health England, Porton Down, UK*
[2] *Laboratory for Zoonoses and Environmental Microbiology, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands*

SUMMARY

This paper reports the first detection of *Borrelia miyamotoi* in UK *Ixodes ricinus* ticks. It also reports on the presence and infection rates of *I. ricinus* for a number of other tick-borne pathogens of public health importance. Ticks from seven regions in southern England were screened for *B. miyamotoi*, *Borrelia burgdorferi sensu lato* (*s.l.*), *Anaplasma phagocytophilum* and *Neoehrlichia mikurensis* using qPCR. A total of 954 *I. ricinus* ticks were tested, 40 were positive for *B. burgdorferi s.l.*, 22 positive for *A. phagocytophilum* and three positive for *B. miyamotoi*, with no *N. mikurensis* detected. The three positive *B. miyamotoi* ticks came from three geographically distinct areas, suggesting a widespread distribution, and from two separate years, suggesting some degree of endemicity. Understanding the prevalence of *Borrelia* and other tick-borne pathogens in ticks is crucial for locating high-risk areas of disease transmission.

INTRODUCTION

Owing to their known vector status, the distribution and geographical expansion of British ticks and the prevalence of pathogens they transmit are important to public and veterinary health. One particularly important tick species is *Ixodes ricinus*, the most commonly reported species to feed on humans in Britain [1]. *I. ricinus* is an important vector of *Borrelia burgdorferi sensu lato* (*s.l.*), the aetiological agent of Lyme borreliosis, which is suggested to have been present within the British tick population for over 100 years [2]. Although there are about 1000 confirmed cases of Lyme borreliosis each year in England and Wales, it is assumed that as many as 3000 cases occur annually, with case numbers steadily increasing since 2001 [3]. Three pathogenic geno-species, *B. burgdorferi sensu stricto* (*s.s.*), *B. afzelii* and *B. garinii* are generally recognized as a cause of Lyme borreliosis worldwide, and all three occur in Britain [3]. *B. garinii* has been associated with neuro-borreliosis and *B. afzelii* and *B. burgdorferi s.s.* have been associated with skin manifestations [4].

Other tick-borne pathogens have also been detected in British tick species, including *Anaplasma phagocytophilum*, *Babesia* spp. [5] and *Rickettsia* spp. [6]. The former, also transmitted by *I. ricinus*,

* Author for correspondence: Miss K. M. Hansford, Medical Entomology & Zoonoses Ecology Group, MRA&BS, Emergency Response Department, Public Health England, Porton Down, UK. (Email: kayleigh.hansford@phe.gov.uk)

is the causative agent of human granulocytic anaplasmosis. It can also cause disease in ruminants and companion animals, and like *Borrelia* bacteria, the prevalence of infection in questing ticks varies between and within countries across Europe [7]. *A. phagocytophilum* is suggested to be widespread within the tick population across the UK causing infection in domestic animals and livestock [8]; however, human cases are rarely reported. *Neoehrlichia mikurensis* has recently been recognized as a cause of tick-borne disease in humans in Europe, although so far it has not been detected in British ticks [9]. Both of these pathogens are suggested to have the potential to interact with *B. burgdorferi s.l.* and through co-infection may affect transmission cycles within nature, and also affect immune responses to infection, resulting in altered disease presentation [10, 11].

More recently however, *B. miyamotoi* has been identified as a spirochaete that can cause relapsing fever in humans. It is related to, but distinct from *B. burgdorferi s.l.*, being phylogenetically more similar to relapsing fever-like *Borrelia* such as *B. lonestari* and *B. theileri* [12, 13]. It was first isolated from ticks in 1995 in *I. persulcatus* from Japan [14] and then later in other Ixodid tick species in North America [15], Canada [16] and Europe. *B. miyamotoi* bacteria have since been found in *I. ricinus* in at least eight different European countries [13, 17–22] where prevalence rates have varied between 0·5% and 5%, and tend to be tenfold less than that of *B. burgdorferi s.l.* [23, 24].

B. miyamotoi has only recently been recognized as a cause of disease in humans, with cases reported from Europe [19], the USA [24–26] and Russia [27]; the latter estimating that 1000 cases per year may occur. A potentially severe complication of *B. miyamotoi* infection is meningoencephalitis. Two different cases have been reported in immunocompromised individuals but not yet in immunocompetent individuals [19, 25]. Immunocompetent patients have a varied clinical presentation, but how this compares with Lyme borreliosis or other tick-borne bacterial infections requires further investigation [24].

Although previous studies have detected the presence of *B. burgdorferi s.l.* and *A. phagocytophilum* in *I. ricinus* from the UK, none have so far detected *B. miyamotoi* and few studies have assessed the prevalence of *A. phagocytophilum* or *N. mikurensis* in questing *I. ricinus*. As part of ongoing risk assessments for the implications of emerging tick-borne pathogens, it is necessary to conduct field and laboratory studies to detect novel and emerging pathogens in British ticks. Using qPCR and DNA sequencing, this study tested *I. ricinus* ticks from a number of locations across southern England for the detection of *B. miyamotoi*. Ticks were collected at different times of year to maximize the detection of *B. miyamotoi*, taking into account possible seasonal variation. Stored ticks were also tested from a previous year to test whether the pathogen may be endemic. Ticks were also screened for *B. burgdorferi s.l.*, *A. phagocytophilum* and *N. mikurensis*. Although it was not an intention of this study to assess prevalence rates for any of these pathogens, some initial assessment of infection rates were determined to guide further field studies.

METHODS

Study areas and tick collection

Questing *I. ricinus* ticks were collected by dragging a 1×1 m cloth over vegetation at various locations across southern England. A sample of 110–240 *I. ricinus* ticks were collected from seven regions (total ~950) including: (*a*) woodland edge and grassland sites in the urban fringe of the city of Salisbury, Wiltshire (51·1° N, 1·8° W), (*b*) four woodland sites (Langley, Redlynch, Whiteparish, Hamptworth) in the northern part of the New Forest National Park (51·0° N, 1·7° W), (*c*) two woodland (Yarner, Dunsford) and two moorland (Shaugh Prior, Burrator near Yelverton) sites in east and west Dartmoor National Park, Devon (50·6° N, 3·7° W and 50·4° N, 4·0° W, respectively), (*d*) four woodland sites (Bentley, Pitton, Grovely, Sutton Mandeville) in south Wiltshire (51·1° N, 1·7° W and 51·1° N, 1·9° W), (*e*) woodland edge and bracken-dominated sites in Richmond Park, London (51·4° N, 0·3° W), (*f*) conifer plantations in Swinley Forest, Berkshire (51·4° N, 0·7° W) and (*g*) woodland/moorland edge sites near Luccombe in Exmoor National Park, Somerset (51·2° N, 3·6° W). In order to maximize the chances of detecting *B. miyamotoi*, *I. ricinus* ticks were collected from six of the regions during the active season from March to August 2013. In addition *I. ricinus* collected from Dartmoor during March–May 2009 were also tested, to give an indication of the presence of *B. miyamotoi* during the last 5 years. Focus was given to nymph and adult ticks as opposed to larvae, owing to the variability of infection rates in this stage [17]. The number of ticks collected

by stage from each site is detailed in Table 1. Ticks were identified using a published taxonomic key [28] and were stored at −80 °C prior to transportation to RIVM.

DNA extraction and PCR amplification

All questing ticks (111 adults, 841 nymphs and two larvae) collected were analysed individually. DNA extraction was carried out on unfed ticks using ammonium hydroxide (NH₄OH) [9]. Next, 100 μl of NH₄OH (1 M) was added to each tick which was then boiled in a heating block at 100 °C for 20–30 min. Tubes were then centrifuged and heated again at 100 °C with the lids off to evaporate the ammonium. The remaining 50 μl lysates were then stored at 4 °C.

Detection of *B. burgdorferi s.l.* and *B. miyamotoi* was carried out using multiplex qPCR in the IQ Multiplex Powermix with a final volume of 20 μl, containing iTaq DNA polymerase (Bio-Rad Laboratories, USA), 200 nM each primer, and 5 μl template DNA [29]. For *B. burgdorferi s.l.* two targets were used, namely the outer surface protein A gene (*OspA*) (forward primer: 5′-AAT ATT TAT TGG GAA TAG GTC TAA-3′; reverse primer: 5′-CTT TGT CTT TTT CTT TRC TTA CA-3′ and probe: 5′-Atto520-AAG CAA AAT GTT AGC AGC CTT GA-BHQ1-3′) and the *Borrelia* flagellin gene (*flaB*) (forward primer: 5′-CAG AIA GAG GTT CTA TAC AIA TTG AIA TAG A-3′; reverse primer: 5′-GTG CAT TTG GTT AIA TTG YGC-3′ and probe: 5′-Atto425-CAA CTI ACA GAI GAA AXT AAI AGA ATT GCT GAI CA-Pho-3′, where X stands for an internal BHQ-1 quencher attached to thymine). A specific part of the *flaB* gene was used for the detection of *B. miyamotoi*, (forward primer: 5′-AGA AGG TGC TCA AGC AG-3′; reverse primer: 5′-TCG ATC TTT GAA AGT GAC ATA T-3′ and probe: 5′-Atto647N-AGC ACA ACA GGA GGG AGT TCA AGC-BHQ2-3′). The multiplex qPCR cycling program (using a light cycler 480 real-time PCR system, Hoffmann–La Roche, Switzerland) was performed using a two-step PCR program: *Taq* activation for 5 min at 95 °C followed by 60 cycles of 5 s at 94 °C and 35 s at 60 °C involving a single point measurement at 60 °C with corresponding filters, finishing with one cycle of 20 s at 37 °C for cooling the plate. Detection of *A. phagocytophilum* and *N. mikurensis* was carried out in a duplex qPCR exactly as described previously [9, 30].

Validation of runs included checking and verifying controls, amplification curves and fluorescence scale and analysis was performed using a second derivative calculation. DNA extraction, PCR master mix preparation, sample addition and PCR amplification were all performed in separate assigned laboratories to minimize cross contamination. Positive and negative controls were also included in each PCR cycle to highlight potential contamination and false positives.

In order to confirm presence and to carry out genospecies analysis, a further PCR was performed on positive tick lysates targeting the *glpQ* (forward primer: 5′-ATG GGT TCA AAC AAA AAG TCA CC-3′; reverse primer: 5′-CCA GGG TCC AAT TCC ATC AGA ATA TTG TGC AAC-3) and *p66* (forward primer: 5′-GAT ACT AAA TTA TTA AAT CCA AAA TCG-3; reverse primer 5′-GGA AAT GAG TAC CTA CAT ATG G-3) genes for *B. miyamotoi* and the 5S-23S intergenic spacer region for *B. burgdorferi s.l.* (forward primer: 5′-GAG TTCGCGGGAGAGTAGGTTATTGCC-3′; reverse primer: 5′-TCAGGGTACTTAGATGGTTCACTT CC-3′), respectively.

Both PCRs for *Borrelia* detection were conducted using Hot Star Taq Master Mix kit (Qiagen, The Netherlands.) The PCR program for *B. miyamotoi glpQ* and *p66* was as follows: *Taq* polymerase activation for 15 min at 95 °C followed by 10 cycles of 30 s at 94 °C, 30 s at 62 °C (annealing temperature, reducing by 1 °C each cycle) and 60 s at 72 °C; then 40 cycles at an annealing temperature of 53 °C; finishing with 10 min at 72 °C. For amplifying the 5S-23S DNA of *B. burgdorferi s.l.* the following program was used: *Taq* polymerase activation for 15 min at 94 °C followed by 10 cycles of 20 s at 94 °C, 30 s at 70 °C (annealing temperature, reducing by 1 °C each cycle) and 30 s at 72 °C; then 40 cycles at an annealing temperature of 60 °C; finishing with 7 min at 72 °C. PCR product was then visualized on agarose gel (1·5%).

DNA sequencing

Both strands of the PCR amplicons of *B. miyamotoi* and *B. burgdorferi s.l.* were sequenced on the Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, USA) using Big Dye Terminator v. 3.1 Cycle Sequencing kit (PerkinElmer, Applied Biosystems). The performed sequences were analysed and compared with sequences available from

Table 1. *Detection of* Borrelia miyamotoi *(Bm),* B. burgdorferi *s.l. (Bb) and* Anaplasma phagocytophilum *(Ap) by tick stage (adults/nymphs), location and sampling period.*

Region	Locations	Habitats	Date	Adults	Nymphs	Total ticks	Bb +ve (% prevalence; exact binomial 95% CI nymphs)	Bm +ve (% prevalence; exact binomial 95% CI nymphs)	Ap +ve (% prevalence; exact binomial 95% CI nymphs)
Wiltshire woodlands	Bentley	Woodland	Mar.–Aug. 2013	13	107	120	2n, 1♀ (1·9%; 0·23–6·6)	0 (0%; 0–4·2)	2n, 2♀ (1·9%; 0·23–6·6)
	Sutton Mandeville		Apr. 2013	0	30	30	0 (0%; 0–11·6)	0 (0%; 0–11·6)	0 (0%; 0–11·6)
	Pitton		Apr. 2013	0	30	30	0 (0%; 0–11·6)	0 (0%; 0–11·6)	0 (0%; 0–11·6)
	Grovely		Apr. 2013	12	48	60	1n (2·1%; 0·05–11·1)	0 (0%; 0–8·9)	0 (0%; 0–8·9)
Exmoor	Luccombe	Wooded moorland	Aug. 2013	7	111*	120	11n, 1♂ (9·9%; 5–17·0)	0 (0%; 0–3·3)	3n (2·7%; 0·56–7·7)
New Forest	Hamptworth	Woodland	Apr. 2013	0	30	30	1n (3·3%; 0·08–17·2)	0 (0%; 0–11·6)	0 (0%; 0–11·6)
	Langley			0	26	26	0 (0%; 0–13·2)	1n (3·8%; 0·1–19·6)	2n (7·7%; 0·95–25·1)
	Redlynch			0	30	30	0 (0%; 0–11·6)	0 (0%; 0–11·6)	0 (0%; 0–11·6)
	Whiteparish			0	34	34	0 (0%; 0–10·3)	0 (0%; 0–10·3)	1n (2·9%; 0·07–15·3)
London	Richmond Park	Wooded parkland	Aug. 2013	37	83	120	0 (0%; 0–4·4)	0 (0%; 0–4·4)	1n, 1♀ (1·2%; 0·03–6·53)
Surrey	Swinley	Pine woodland	Aug. 2013	20	94	114	2n, 2♀ (2·1%; 0·3–7·5)	0 (0%; 0–3·9)	0 (0%; 0–3·9)
Salisbury	Urban fringe	Woodland edge and grassland	May–July 2013	9	111	120	7n, 3♀ (6·3%; 2·6–12·6)	1n (0·9%; 0·02–4·9)	0 (0%; 0–3·3)
Dartmoor	Dunsford	Woodland	Mar.–May 2009	6	0	6	0 (0%; 0–45·9)	0 (0%; 0–45·9)	0 (0%; 0–45·9)
	Bovey Tracey	Woodland		2	59	61	8n (13·6%; 6·0–25·0)	0 (0%; 0–6·1)	7n (11·9%; 4·9–22·9)
	Yelverton	Moorland		5	48	53	1n (1·9%; 0·05–11·1)	1n (2·1%; 0·05–11·1)	3n (6·3%; 1·3–17·2)
Total				111	841	954	33n, 6♀, 1♂ (3·9%; 2·7–5·5)	3n (0·4%; 0·07–1·0)	19n, 3♀ (2·3%; 1·4–3·5)

* Two larvae were also tested but were negative.

n, ♀, ♂ = nymph, female, male, respectively.

Exact binomial 95% confidence intervals (CIs) for nymphs infected with *Borrelia burgdorferi s.l.* (Bb), *B. miyamotoi* (Bm) and *Anaplasma phagocytophilum* (Ap) are displayed in parentheses for each location and also the total nymphs collected.

Fig. 1. Locations of field collections of questing *Ixodes ricinus* ticks in southern England.

Genbank with the use of Bionumerics software (Applied Maths NV, Belgium). *A. phagocytophilum* was not sequenced.

Statistics

Minitab v. 16 (State College, PA, USA) was used to generate exact binomial 95% confidence intervals (CIs) for pathogen prevalence rates and to perform a two-tailed Fisher's exact test to compare *B. burgdorferi s.l.* and *B. miyamotoi* prevalence.

RESULTS

B. miyamotoi was detected in three of the 954 ticks tested (0·3%, 95% CI 0·06–0·92). All positive ticks were nymphal *I. ricinus* and were collected from three geographically distinct locations during April 2013 in the New Forest, May 2013 in urban Salisbury and April 2009 in west Dartmoor, suggesting a wide geographical distribution (Fig. 1). DNA amplification with conventional PCR of one of the three *B. miyamotoi* isolates was successful. Analysis of both *glpQ* and *p66* genes showed clustering with other isolates from Europe (Fig. 2).

B. burgdorferi s.l. was detected in 40/954 ticks (4·2%, 95% CI 3·01–5·7) tested, with at least one positive tick collected from all locations surveyed, except Richmond Park, London. Infected ticks were collected during March, April and May in 2009 and during April, May, July and August in 2013. *B. burgdorferi s.l.* was detected in 33/841 nymphs tested (4%, 95% CI 2·7–5·5), 6/90 females (6·6%, 95% CI 2·5–14·0) and 1/21 males (5%, 95% CI
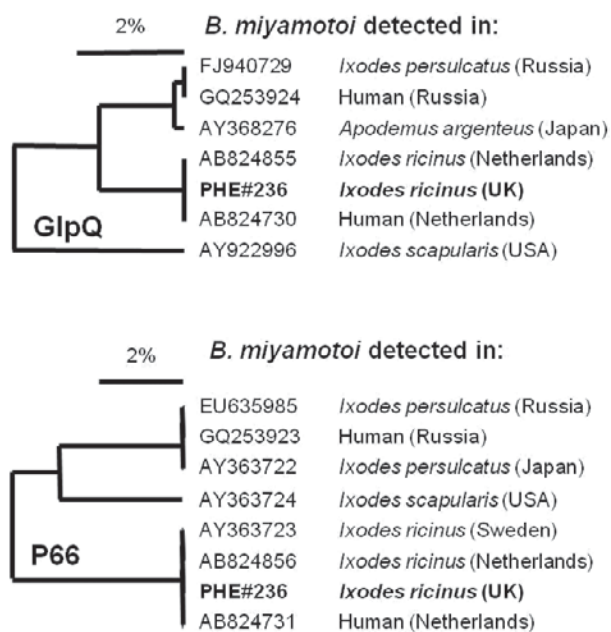

Fig. 2. Phylogenetic tree of UK *Borrelia miyamotoi* isolate and several reference sequences from Genbank.

0·12–23·8). The highest numbers of infected ticks were found in Exmoor (10%, 95% CI 5·3–16·8), Salisbury (8·3%, 95% CI 4·1–14·8) and Dartmoor (7·5%, 95% CI 3·5–13·8) (Table 1).

Twenty-four ticks yielded sequence products corresponding to *B. burgdorferi s.l.* Twelve sequences were matched to *B. garinii* isolates, seven to *B. afzelii* and five to *B. valaisiana* (Table 2). All potential pathogenic isolates from Exmoor and Salisbury were *B. garinii* and all potential pathogenic isolates from Wiltshire woodlands and Surrey were *B. afzelii*.

Table 2. *Summary of genospecies of* Borrelia *DNA sequencing by location*

Region	B. afzelii	B. garinii	B. valaisiana	No sequence data
Wiltshire woodlands	2	0	1	1
Exmoor	0	6	1	5
New Forest	0	0	0	1*
Surrey	3	0	1	0
Salisbury	0	2	1	7
Dartmoor	2	4	1	2
Total	7	12	5	16

* *B. miyamotoi* was sequenced from an isolate in the New Forest.

Both pathogenic genospecies were isolated from ticks from Dartmoor.

A. phagocytophilum was detected in 22/954 ticks (2·3%, 95% CI 1·5–3·5) collected from five of the regions surveyed (Table 1). Infected ticks were collected during March and April in 2009 and April, May, June, July and August during 2013. *A. phagocytophilum* was detected in 19/841 nymphs tested (2·3%, 95% CI 1·4–3·5) and 3/90 females (3·3%, 95% CI 0·69–9·43), with the highest numbers of infected ticks found in Dartmoor (8·3%, 95% CI 4·1–14·8) (Table 1). One co-infection of *B. afzelii* and *A. phagocytophilum* was detected in a female *I. ricinus* in a Wiltshire woodland (Bentley) during May 2013. No other co-infections were detected, despite the presence of at least two different pathogens within ticks collected from five of the seven regions surveyed. DNA of *N. mikurensis* was not detected in any of the 954 tick lysates.

DISCUSSION

The detection of potential human pathogens in ticks and the need to understand their ecological drivers is essential for helping to protect public health from emerging tick-borne pathogens; particularly those that may cause similar symptoms to endemic diseases [31]. This is the first detection of *B. miyamotoi* in *I. ricinus* ticks in the UK. There is no evidence so far to suggest that this pathogen causes human clinical disease in the UK, however it highlights that a number of non-Lyme tick-borne potential pathogens occur in UK ticks. There is sufficient evidence from other countries to suggest that *B. miyamotoi* may cause human disease, and further investigations of this bacterium as a potential cause of clinical disease in the UK are recommended.

Ticks infected with *B. miyamotoi* were from three geographically distinct areas of southern England; in a rural wooded habitat in the New Forest, in an urban woodland habitat in Salisbury, and from a moorland habitat in Dartmoor. This suggests a possible widespread distribution of *B. miyamotoi* infected ticks across southern England and that a range of ecologically diverse habitats may support the transmission of this pathogen. Positive ticks were collected during 2009 and 2013 suggesting some degree of endemicity. It is feasible that *I. ricinus* collected before 2009 may test positive for *B. miyamotoi*, considering that the pathogen has been found previously in *I. ricinus* ticks elsewhere in Europe, perhaps as early as 1986 [32]. Future studies that broaden the geographical range, and focus on positive sites will provide further information to better assess the distribution and prevalence rates of *B. miyamotoi*.

B. burgdorferi s.l. infection rates in ticks was significantly higher compared to *B. miyamotoi* (Fisher's exact, $P = 0.0001$), and this mimics other studies where a tenfold higher rate of *B. burgdorferi s.l.* has been reported [23, 31]. Forty ticks tested positive for *B. burgdorferi s.l.* (4·2%, 95% CI 3·01–5·7), which is similar to overall prevalence rates found in other studies in the UK [33–35] which reported 3·3%, 5–7·7% and 5·6%, respectively. However, direct comparisons between prevalence rates need to be made with caution due to differences in sample sizes, stages of ticks tested and molecular methods used.

Twenty-four of the 40 positive samples were successfully genotyped, with *B. garinii* being the most commonly reported (50%), followed by *B. afzelii* (29%) and *B. valaisiana* (20%) (Table 2). Although low numbers of infected ticks were sequenced (but within the expected levels for the molecular method used), this finding is in contrast to a previous study in northern England where *B. valaisiana* (58%) and *B. garinii* (33%) were reported as the most commonly detected genotypes [33] and also to a study in Scotland which reported *B. afzelii* (48%) and *B. garinii* (36%) as the most common [35]. *B. burgdorferi s.l.*-infected ticks were found in all survey sites except Richmond Park; however, infected ticks have been reported here previously [36].

Although a range of habitats suitable for ticks were surveyed, the number of infected ticks between sites varied, with sites supporting high tick densities not necessarily being supportive of high numbers of ticks infected with *B. burgdorferi s.l.* This may be explained by the complex ecology of *B. burgdorferi s.l.*, but further work is required here. A number of studies from Europe [37, 38] have reported high *B. burgdorferi s.l.* infection rates in ticks in urban areas and associated this with an underestimated public health risk [39]. This may also be the case for ticks in urban Salisbury where 8·3% (95% CI 4·1–14·8) of ticks were found to be infected. However, further field data is required from Salisbury and from additional urban sites to investigate the significance of such habitats with regards to Lyme borreliosis risk. Interestingly this urban site was also one of the three sites harbouring *B. miyamotoi*-infected ticks.

It is noteworthy that in four of five regions with sequence data, only one pathogenic genospecies of *B. burgdorferi s.l.* was detected in each, suggesting, albeit with low sample sizes, a dominance of different genotypes within different habitats. This again may be explained by the ecology of the pathogen, and further studies that investigate the spatial heterogeneity of genospecies infection rates may help to guide the understanding of the clinical disease in humans.

A. phagocytophilum was detected in 2% (95% CI 1·4–3·5) of nymphs and 3% (95% CI 6·9–9·4) of females, and in accordance with *B. burgdorferi s.l.* and *B. miyamotoi*, ticks infected with *A. phagocytophilum* were found across all regions except Surrey and Salisbury. The detection of *A. phagocytophilum* and *B. afzelii* co-infection in one of 90 females tested is also worth noting. Although this was a rare occurrence in our study, the cross-over of the distribution of *B. burgdorferi s.l.* and *A. phagocytophilum* infected ticks in five of the seven regions surveyed, highlights the opportunity for co-infection in humans. This is particularly important as co-infection in humans is known to affect human susceptibility to infection with *B. burgdorferi s.l.* [10] with both pathogens acting synergistically to avoid host immune responses. This can result in mixed clinical manifestations (making diagnosis more difficult) and increased disease severity [11].

N. mikurensis is a potentially emerging tick-borne pathogen, having caused febrile illness in a number of immunocompromised humans since 2010 [9] and may also interact with other tick-borne pathogens such as *B. burgdorferi s.l.* [10]. It was not detected in any of the 954 tick lysates tested in our study, suggesting, along with evidence from a previous study [9], that it may not be established within UK tick populations. The presence of this pathogen in *I. ricinus* in many European countries has been well documented [9, 40–42] making the apparent absence of this pathogen in UK ticks of interest to both UK and European researchers.

Unlike *B. burgdorferi s.l.*, transovarial transmission is highly probable in *B. miyamotoi*, with some studies suggesting over 90% of larvae derived from *B. miyamotoi*-infected egg batches were infected [17]. Furthermore, the numbers of *Borrelia* bacteria are reported to be significantly higher in ticks infected with *B. miyamotoi* compared to *B. burgdorferi s.l.* [22]. High transovarial transmission could result in more persistent infection of larvae in the environment, and this could pose a potential health risk should they bite people and transmit *B. miyamotoi* [43]. This may be important as human bites by larval *I. ricinus* are currently considered less important in *B. burgdorferi s.l.* transmission. The role of wildlife in the transmission *of B. miyamotoi* is currently unknown and requires further investigation, as well as the role of other human-biting tick species, such as *I. hexagonus*; the second most common tick species reported to bite humans in the UK.

It is important to recognize that this study was purely to detect the presence of pathogens, specifically *B. miyamotoi*. Further studies are now required to assess the prevalence of this and other tick-borne pathogens in ticks in England. Furthermore, infection rates and tick activity vary spatially and temporally and therefore snapshot infection rates should be treated with caution. Nevertheless they are useful in guiding further field studies in *Borrelia* and *Anaplasma* endemic areas. They also improve our understanding of the distribution of pathogens as well as the possible infection rates of pathogens in ticks.

This study presents the first detection of *B. miyamotoi* in British ticks, with the spirochaete detected in ticks from a wide geographical area in both rural and urban locations in southern England. The public health significance of *B. miyamotoi* has been debated elsewhere [19], [31], but published evidence suggests that it does cause clinical disease, and further studies that investigate this in the UK are recommended. Moreover, there is uncertainty regarding the detection of *B. miyamotoi* infections using current diagnostic tests for Lyme borreliosis, therefore this requires further investigation [19, 24, 25, 31].

Further research which allows us to establish prevalence rates and potential reservoir hosts of all three pathogens within the surveyed regions, and elsewhere in the UK, should be prioritized in order to further understand the ecological drivers of the diseases they cause. Raising awareness of *B. miyamotoi* among public health professionals and researchers alike will help to define the clinical picture of disease caused by *B. miyamotoi*. It may also encourage the consideration of co-infections during differential diagnosis to support the further quantification of the risk of tick-borne disease to UK public health.

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DECLARATION OF INTEREST

None.

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