Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Prevalence of *Borrelia burgdorferi* and *Borrelia miyamotoi* in questing *Ixodes ricinus* ticks from four sites in the UK

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ARTICLE INFO

Keywords:
Borrelia burgdorferi sensu lato
Borrelia miyamotoi
Ixodes ricinus
Lyme borreliosis

ABSTRACT

Borrelia miyamotoi is a spirochete bacterium related to *Borrelia burgdorferi* sensu lato, the cause of Lyme borreliosis, and vectored by ticks. In 2014, *B. miyamotoi* was identified in three questing *Ixodes ricinus* collected in the UK. We sought to confirm the presence of *B. miyamotoi* in the UK. Ticks were collected from four locations not previously investigated for *B. miyamotoi* or *B. burgdorferi* s.l. and of which two are considered as Lyme borreliosis "hotspots" based on hospital records of the disease. We independently confirm that *B. miyamotoi* is present in the UK and support the view that *B. miyamotoi* is likely to have a broad geographic distribution, at low levels. Our study also adds to the existing data on the distribution of *B. burgdorferi* s.l. in the UK and demonstrates that although the two "hotspots" had relatively high tick densities, they did not have the highest proportion of infected ticks.

1. Introduction

Ticks transmit various pathogens that cause zoonoses, one of which is Lyme borreliosis, caused by the spirochete *Borrelia burgdorferi* sensu lato (s.l.)-complex. In Europe, the hard tick, *Ixodes ricinus* is the most common vector of these tick-borne pathogens. The *B. burgdorferi* s.l. complex comprises a number of genospecies, the most important ones in the UK being *B. burgdorferi* sensu stricto (*B. burgdorferi* s.s.), *B. garinii* and *B. afzelii*. In humans, these commonly cause erythema migrans, fever, arthralgia, myalgia and fatigue (Dubrey et al., 2014). The number of recorded cases of Lyme borreliosis in England and Wales has been steadily rising from 268 in 2001–959 in 2011, an increase of 258% (Public Health England, 2013). In addition, up to 2000 cases annually are thought to go undiagnosed (British Infection Association, 2011). There are several possible reasons which have been put forward to explain this increase such as the increased sensitivity of diagnostic tests, the increased availability of diagnostic services and greater awareness of Lyme borreliosis symptoms (Public Health England, 2013). It has also been suggested that tick populations have increased as a result of increases in deer populations (Scharlemann et al., 2008; Gilbert et al., 2012). Although deer can clear *B. burgdorferi* s.l. from ticks (Roome et al., 2017), it has been shown that deer contribute to increases in Lyme borreliosis risk (Mysterud et al., 2016). High deer populations allow larger numbers of adult female ticks to feed successfully and subsequently produce larger numbers of larval progeny (Millins et al., 2017). Since larvae and nymphs preferentially feed on small mammal and bird hosts, which act as disease reservoirs; this causes an increase in infected nymph density (Millins et al., 2017).

Several regions in the UK have been classed as tick and Lyme borreliosis "hotspots" based on areas understood to have a high tick density and where many cases of the disease have been reported. These include Exmoor, the Lake District, Thetford, New Forest, Salisbury Plain, the South Downs, West Sussex, Surrey, West Berkshire, Wiltshire, Yorkshire moors, Scottish Highlands, Richmond Park (London) and Bushy Park (London) (Dubrey et al., 2014). Although the tick infection prevalence in questing ticks in some of these hotspots has been examined (Davidson et al., 1999; Kurtenbach et al., 2001; Vollmer et al., 2011; James et al., 2013; Hansford et al., 2015), others have not been investigated. Data on questing tick density and tick infection prevalence are necessary to identify which areas pose most risk to individuals, rather than identifying these areas based on the number of Lyme borreliosis cases, which is dependent on visitor numbers.

A species related to *B. burgdorferi* s.l., known as *Borrelia miyamotoi*, has been reported to cause illness similar to Lyme borreliosis (Platonov et al., 2011). *B. miyamotoi* was first recorded in Japanese *Ixodes* ticks in 1995 (Fukunaga et al., 1995) and has since been found in ticks collected in Russia (Platonov et al., 2011), the USA (Ullmann et al., 2005; Hamer et al., 2014), Canada (Ogden et al., 2011; Dibernardo et al., 2014) and

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http://dx.doi.org/10.1016/j.ttbdis.2017.09.007
Received 25 April 2017; Received in revised form 14 September 2017; Accepted 14 September 2017

parts of Europe (Wilhelmsson et al., 2010). In immunocompetent individuals, *B. miyamotoi* causes influenza-like symptoms including relapsing fever, headache, nausea, fatigue, myalgia and arthralgia (Branda and Rosenberg, 2013). Although *B. miyamotoi* infection is frequently referred to as a relapsing fever, it has been argued that it should be termed "*Borrelia miyamotoi* disease" since the symptoms are less severe than a tick-borne relapsing fever (Telford et al., 2015). In immunocompromised individuals, more severe symptoms such as meningoencephalitis have been noted (Gugliotta et al., 2013; Hovius et al., 2013). In 2014, *B. miyamotoi* was recorded in three nymphal *I. ricinus* ticks collected in the UK (Hansford et al., 2015) but this observation has yet to be independently confirmed. Human infections caused by *B. miyamotoi* in the UK have not been reported or identified so understanding the geographical prevalence of this organism in UK ticks can assist in assessing risk and likelihood of infection.

Besides investigating if *B. miyamotoi* is present in questing ticks in the UK, the aims of this study were to compare the tick density and prevalence of tick infection with *B. burgdorferi* s.l. and *B. miyamotoi* between two tick and Lyme borreliosis "hotspots" (Thetford Forest and West Dean), a location understood to have a high tick density (Cirencester Park), and a location where *I. ricinus* has not established despite a favourable habitat (Wytham Woods). These locations were chosen because they have not been investigated previously and they are separated spatially by at least 45 km, covering different regions of England. Questing ticks were collected by blanket dragging at each of the four locations during spring 2014 and screened for *Borrelia* by multiplex quantitative PCR. *Borrelia* genospecies of positive samples were identified by DNA sequencing.

2. Materials & methods

2.1. Tick collection

Ticks were collected from 4 locations in the United Kingdom (Fig. 1): Cirencester Park, Gloucestershire (51.713163°N,–2.060698°W) on 18th March 2014; West Dean, South Downs National Park, West Sussex (50.939146°N,–0.786328°W) on 26th March 2014; Kings Forest, Thetford Forest, Suffolk (52.351393°N,0.676978°W) on 9th April 2014, and Wytham Woods, Oxfordshire (51.774881°N,–1.331728°W) on 14th April 2014. Weather conditions were dry with temperatures between 8 and 18 °C. At each location, 2 woodland habitat sites and 2 ecotone habitat sites adjacent to woodland were surveyed using the blanket dragging method (Macleod, 1932). For Thetford and West Dean, one woodland site is deciduous and the other evergreen whereas for Cirencester and Wytham Woods both woodland sites are deciduous. The blanket was 1.5 m wide and 1.85 m long (2.775 m^2). It was dragged 6 m and then checked for ticks. Any ticks found were placed inside a 1.5 ml Eppendorf tube. This was repeated 10 times at each site resulting in 60 m total distance dragged at each site. However, for Cirencester, 5 sites were sampled of which 3 were ecotone and 2 were woodland. The first site (A) was dragged in 20 × 6 m repeats resulting in 120 m of habitat sampled. For Site B there were 5 × 6 m repeats. Ticks were stored at −80 °C until DNA extraction.

2.2. DNA extraction

DNA for PCR analysis was extracted from the ticks, using an ammonium hydroxide lysis method, adapted from Guy and Farquhar, 1991. The ticks were placed individually into the wells of a 96 well plate. 100 µl of 1 M ammonium hydroxide (Sigma Aldrich) was pipetted into each well. Negative extraction controls were incorporated into the extraction process. The plate was sealed and placed in a PCR thermal cycler at 99 °C for 20 min to lyse the samples. After lysis, the plate was briefly centrifuged at 1000 rpm and the plate seal was removed. The plate was then incubated at 99 °C for a further 20 min to evaporate the ammonia. Approximately 50 µl of solution remained. The samples were stored at −20 °C until required.

2.3. Detection of Borrelia burgdorferi sensu lato and Borrelia miyamotoi

Multiplex qPCR was used to determine which tick extracts contained *B. burgdorferi* s.l. and *B. miyamotoi* according to the method by Hansford et al., 2015 (refer to this paper for primer and probe sequences). Each well of the PCR plate contained 20 µl comprising 400 nM B-OspA_modF primer, 400 nM B-OspA_borAS primer, 100 nM B-OspA_mod-probe, 200 nM B-FlaB-F primer, 100 nM B-FlaB-Rc primer, 100 nM B-FlaB-Rt primer, 200 nM B-FlaB-FAM probe, 200 nM FlabBm.motoiF primer, 200 nM FlabB.m.motoiR primer, 200 nM FlabBm.motoiP primer, 10 µl 2x iQ multiplex Powermix (Bio-rad) and 5 µl sample. Molecular grade water was used as a negative control and *B. burgdorferi* s.s. DNA as a positive control. The PCR was performed using the Applied Biosystems 7500 Fast Real-time PCR Machine. The program consisted of 5 min at 95 °C followed by 45 cycles of 5 s at 94 °C and 35 s at 60 °C.

To confirm the presence of *B. miyamotoi* in the samples PCR positive for *B. miyamotoi*, the *glpQ* gene was amplified and sequenced using the method by Hansford et al., 2015 (refer to for primer sequences). The PCR program consisted of a Taq activation step at 95 °C for 15 min followed by 94 °C for 30 s (denaturation), 62 °C for 30 s (annealing), lowering by 1 °C per cycle, and 72 °C for 60 s (elongation) for 10 cycles. The program continued for 40 cycles at: 94 °C for 30 s, 53 °C for 30 s and 72 °C for 60 s. There was then a final extension step at 72 °C for 10 min.

The products were then analysed by nucleic acid electrophoresis on a 2% TBE agarose gel. Products were sequenced on a 3130xL Genetic Analyzer using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The computer program SeqTrace (Stucky, 2012) was used to create the consensus sequences from the forward and reverse primer

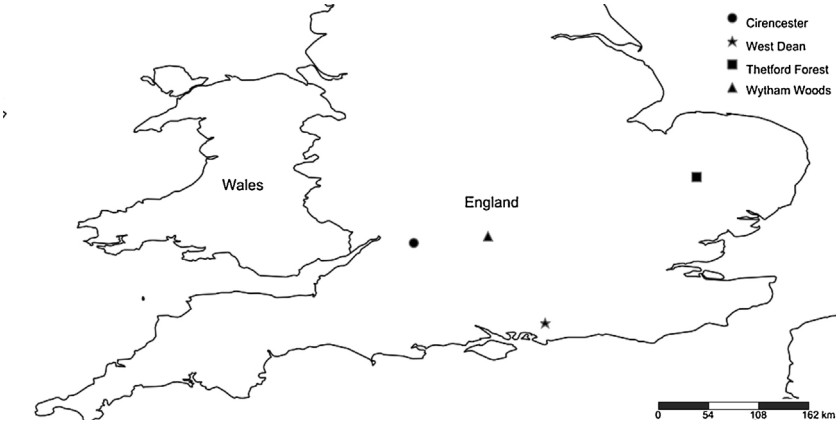

Fig. 1. The 4 locations sampled (Shorthouse, 2010).