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Original article

Ticks and *Borrelia* in urban and peri-urban green space habitats in a city in southern England

Kayleigh M. Hansford[a,c,*], Manoj Fonville[b], Emma L. Gillingham[a], Elena Claudia Coipan[b], Maaike E. Pietzsch[a], Aleksandra I. Krawczyk[b], Alexander G.C. Vaux[a], Benjamin Cull[a], Hein Sprong[b], Jolyon M. Medlock[a,c,d]

[a] *Medical Entomology & Zoonoses Ecology, Emergency Response Department – Science & Technology, Health Protection Directorate, Public Health England, Porton Down, UK*
[b] *National Institute of Public Health and the Environment (RIVM), Laboratory for Zoonoses and Environmental Microbiology, Bilthoven, The Netherlands*
[c] *NIHR Health Protection Research Unit in Environmental Change and Health, UK*
[d] *NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, UK*

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ABSTRACT

Ticks are becoming increasingly recognised as important vectors of pathogens in urban and peri-urban areas, including green space used for recreational activities. In the UK, the risk posed by ticks in such areas is largely unknown. In order to begin to assess the risk of ticks in urban/peri-urban areas in southern England, questing ticks were collected from five different habitat types (grassland, hedge, park, woodland and woodland edge) in a city during the spring, summer and autumn of 2013/2014 and screened for *Borrelia burgdorferi* sensu lato. In addition, seasonal differences in *B. burgdorferi* s.l. prevalence were also investigated at a single site during 2015. *Ixodes ricinus* presence and activity were significantly higher in woodland edge habitat and during spring surveys. DNA of *Borrelia burgdorferi* s.l. was detected in 18.1% of nymphs collected across the 25 sites during 2013 and 2014 and two nymphs also tested positive for the newly emerging tick-borne pathogen *B. miyamotoi*. *Borrelia burgdorferi* s.l. prevalence at a single site surveyed in 2015 were found to be significantly higher during spring and summer than in autumn, with *B. garinii* and *B. valaisiana* most commonly detected. These data indicate that a range of habitats within an urban area in southern England support ticks and that urban *Borrelia* transmission cycles may exist in some of the urban green spaces included in this study. Sites surveyed were frequently used by humans for recreational activities, providing opportunity for exposure to *Borrelia* infected ticks in an urban/peri-urban space that might not be typically associated with tick-borne disease transmission.

1. Introduction

Borrelia burgdorferi sensu lato, the causative agent of Lyme borreliosis, is a tick-borne pathogen of global public health importance. In order to assess risk and develop intervention strategies against such tick-borne diseases, an understanding of tick vector biology, distribution, ecology and importantly the potential for contact with humans are needed (Dobson et al., 2011; Medlock and Jameson, 2010; Sprong et al., 2012). Typically, the risk of acquiring Lyme borreliosis has been associated with deciduous and mixed woodland (Medlock et al., 2012), and as a result, many research studies have been conducted within what would be considered rural habitat or the natural environment. However, it has been shown that green spaces within urban areas can also provide suitable habitat for ticks and within these areas, ticks have tested positive for various tick-borne pathogens, which poses a potential risk to public health (LaDeau et al., 2015; Rizzoli et al., 2014; Uspensky, 2014).

We define urban areas as well developed with a high density of urban structures and highly fragmented environments that consist of habitat patches of varying sizes, vegetation and land-use types. Alongside urban areas, peri-urban habitats fringe the urban areas, and are congruent to the rural areas. Such habitats may have lower biodiversity compared to rural habitat (Rizzoli et al., 2014). It is considered that the use of green space in urban areas is linked to improved human health and well-being (Public Health England, 2014). Urban green spaces are a focal point of UK gov-

* Corresponding author at: Medical Entomology & Zoonoses Ecology, Emergency Response Department – Science & Technology, Health Protection Directorate, Public Health England, Porton Down, UK.
E-mail address: kayleigh.hansford@phe.gov.uk (K.M. Hansford).

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ernment adaptation plans to mitigate the effects of heatwave, air pollution, flooding and other health risks currently associated with our changing climate (Committee on Climate Change, 2014). In addition, green spaces in urban areas can be utilised as part of conservation strategies for wildlife. The benefits of urban green spaces and the arguments for increasing and improving access to them are clear, but it is also important to balance this with raising awareness that these spaces can also occasionally provide opportunity for contact between humans and infected ticks.

The risk of acquiring tick-borne diseases has been measured using various strategies, such as tick abundance within a particular area (Jaenson et al., 2009; Soleng and Kjelland, 2013), thus higher risk has often been associated with rural, deciduous woodland areas where ticks can be found in high numbers. However, pathogen prevalences within ticks do not always correlate with abundance and it is actually the density of infected ticks that is important (Coipan et al., 2013; James et al., 2013; Tack et al., 2011). In addition, high pathogen prevalences of ticks in a rural area where the potential for human contact with ticks might be minimal means that such an area may not actually present a significant risk. On the contrary, an urban green space habitat with lower tick abundance but increased pathogen prevalence and a higher probability of human-tick contact, may mean that the risk posed by ticks in such areas is underestimated (Hansford et al., 2014).

This study investigated the range of habitats that might support ticks in a city (Salisbury) in southern England, focusing on areas that are frequented by humans. The aim of this study was to determine whether green space within an urban/peri-urban area could support ticks and if so, whether those ticks were infected with *Borrelia* bacteria. Additionally, the potential influence of seasonal and ecological factors on tick presence, abundance and *Borrelia* prevalence was also investigated. The potential risk posed by ticks infected with *Borrelia* bacteria within an urban/peri-urban area is also discussed.

2. Methods

2.1. Study sites

Salisbury is an urban area located in Wiltshire, England [human population: ~41,000 according to the most recently available census data (2011)] covering an area of 1924 ha. The city was segregated into urban areas [i.e. the inner part of the city] and peri-urban areas [i.e. the edge of the city where the urban areas were congruent with rural areas]. Green space within Salisbury includes readily accessible and restricted/controlled areas, including parks and public gardens, general amenity space such as village greens and commons, as well as community woodland. Important tick hosts such as woodland birds (e.g. blackbird, robin), wood mice, bank voles, foxes, grey squirrels and hedgehogs, as well as roe deer, are likely to be present in Salisbury.

Satellite imagery from Google Earth and the Land Cover Map 2000 (LCM2000, Centre for Ecology and Hydrology, UK) were used to identify potentially suitable tick survey sites in Salisbury. These sites were then visited to determine the reliability of the satellite imagery (Estrada-Peña, 2002) and site locations were captured using a GPS data logger. Sites were then classified into five categories: grassland, hedge, park, woodland and woodland edge. In total, 25 publicly accessible sites used for recreation were chosen (Fig. 1), as these represented areas where humans and ticks could come into contact (Corrain et al., 2012).

During 2013, all 25 sites were surveyed during a two week period in spring, summer and autumn. During 2014, 23 of the original sites were surveyed during the same time periods but two sites were excluded due to becoming inaccessible (one was developed into housing during the survey, and the other became overgrown with vegetation). During 2015, an additional, separate survey was carried out during the same time periods at one of the initial 25 sites (a woodland edge habitat) to further assess possible seasonal changes in *Borrelia* prevalences. Vegetation at this site was continually surveyed during each season until 50 nymphs were collected for *Borrelia* testing. Surveys were conducted between 10am and 4pm on dry days with average temperatures above 7 °C.

2.2. Tick collections

A 1 m^2 poly-cotton cloth attached to a wooden handle was used to survey for ticks in this study. Ten separate 10 m transects were conducted in each location, to cover a total area of 100 m^2 (Estrada-Peña et al., 2013). The same transects were surveyed at each site during the spring, summer and autumn surveys across the years. Ticks were collected from the cloth, frozen at −80° C and individually identified to species level using a morphological key (Hillyard, 1996). Larval presence was also noted and will be described in the results, but larvae were not counted or collected and were not included in any statistical analysis in this study.

A number of variables and observations were recorded at each transect including; date, time, ambient temperature (measured 1 m above ground level) and relative humidity (measured 1 m above ground level) (Precision Hygro-thermometer, CEM, China), cloud cover (oktas), soil moisture (Theta soil moisture probe and HH2 meter, Delta-T Devices, UK), habitat type (grassland, hedge, park, woodland and woodland edge) vegetation height and mat depth (measured using a 1 m ruler) and type (grass, ivy, nettles, leaf litter or brambles), and whether there were signs of any hosts presence, either through visualising actual hosts or evidence of a host e.g. faeces, animal tracks, birdsong. Human presence along each transect at the time of survey was also noted.

2.3. Pathogen screening

A subset of ticks collected during 2013 and 2014 were analysed individually in 0.2 ml PCR tubes (Estrada-Peña et al., 2013) for the presence of *Borrelia* DNA. The additional 150 nymphs collected during spring, summer and autumn 2015 at the single woodland edge habitat were also screened for *Borrelia* DNA.

All ticks collected were considered to be unfed and DNA extraction was carried out using ammonium hydroxide (NH_4OH) (Jahfari et al., 2012). A total of 100 μl of 1 M NH_4OH was added to each tick, which was boiled in a heating block for 20–30 min. Tubes were centrifuged and heated again to 100° C with the lids off to evaporate the ammonium. Tick lysates were stored at 4° C until PCR screening was carried out.

qPCR and sequencing was carried out as previously described (Hansford et al., 2014). Ticks were screened for *B. burgdorferi* s.l. and *B. miyamotoi* using a multiplex qPCR with IQ multiplex powermix containing iTaq polymerase as previously described. Outer surface protein A (OspA) and *Borrelia* flagellin gene (FlaB) were targeted for *B. burgdorferi* s.l. and a specific part of the flaB gene was targeted for the detection of *B. miyamotoi*. A 480 lightcycler real-time PCR system was used for a two-step PCR program as described previously. Positive and negative controls were included in each PCR cycle. DNA extraction, PCR master mix preparation, sample addition and PCR amplification were each carried out in separate laboratories to eliminate potential cross-contamination. Genospecies analysis of *Borrelia* positive samples was carried out using a further PCR targeting the 5S-23S inter-generic spacer gene for *B. burgdorferi* s.l. To confirm any *B. miyamotoi*-positive samples, a PCR targeting the glpQ and p66 genes was conducted. PCR product was then visu-