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Prevalence of ticks and tick-borne pathogens: *Babesia* and *Borrelia* species in ticks infesting cats of Great Britain

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ABSTRACT

In a study of tick and tick-borne pathogen prevalence, between May and October 2016, 278 veterinary practices in Great Britain examined 1,855 cats. Six-hundred and one cats were found to have attached ticks. The most frequently recorded tick species was *Ixodes ricinus* (57.1%), followed by *Ixodes hexagonus* (41.4%) and *Ixodes trianguliceps* (1.5%). Male cats, 4 - 6 years of age living in rural areas were most likely to be carrying a tick; hair length and tick treatment history had no significant association with attachment. For cats that were parasitized by ticks in large urban areas, *I. hexagonus* was the most frequent species recorded. Molecular analysis was possible for 541 individual tick samples, others were too damaged for analysis; *Babesia* spp., and *Borrelia burgdorferi* sensu lato were identified in 1.1% (n=6) and 1.8% (n=10) of these, respectively. *Babesia* spp. included *Babesia vulpes* sp. nov./*Babesia microti*-like (n=4) in *I. hexagonus* and *Babesia venatorum* (n=2) in *I. ricinus. Borrelia burgdorferi* s.l. species included *Borrelia garinii* (n=6) and *Borrelia afzelii* (n=4). The majority of *B. burgorferi* s.l. cases were found in *I. ricinus*, with *B. afzelii* in one *I. hexagonus* nymph. No *Borrelia* or *Babesia* spp. were present in *I. trianguliceps.* To determine a true prevalence for ticks on cats, practices that only submitted questionnaires from cats with ticks and practices that submitted fewer than 5 returns per week were removed; amongst those considered to have adhered strictly to the collection protocol, feline tick prevalence amongst cats that had access to the outdoors was 6.6%. These results show that ticks can be found on cats throughout Great Britain, which harbour a range of species of *Babesia* and *B. burgdorferi* s.l. and that cats, particularly in green spaces within urban areas, may form an important host for *I. hexagonus*, a known vector of pathogens.

Key words: Ixodidae, Cat, Survey, Distribution, Zoonosis

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1. Introduction

Ticks are an important group of arthropod vectors (Otranto & Wall, 2008) and transmit a wide range of viral, bacterial and protozoan pathogens. For three-host species in particular, their lack of host specificity allows them to feed on a different host in each life cycle stage and enhances their ability to transmit pathogens between host populations. In addition, their role as vectors is exacerbated by large population densities facilitated by high rates of reproduction, and the transmission of the pathogens they carry between the different life cycle-stages (trans-stadial transmission) while co-feeding and between generations via eggs (trans-ovarial transmission). Infection of the host with tick-transmitted pathogens may be aided by salivary anticoagulants and other active compounds that modulate host cutaneous immunity and inflammation, while enhancing vasodilatation to bring more blood to the feeding site (Wikel, 1999). In the future, tick-borne disease may become an increasing concern as the changing climate favours tick survival in new geographical locations; previous studies have recorded correlations between tick population size and higher temperature (Lindgren et al., 2000; Gray et al., 2009; Tagliapietra et al., 2011; Jaenson et al., 2012; Korotkov et al., 2015). Associations between tick density and increasing host abundance have also been shown in multiple studies (Lindgren et al., 2000; Gilbert et al., 2012; Abdullah et al., 2016; Cat et al., 2017).

Companion animals are abundant, represent an easily available food source for ticks and may act as a reservoir for pathogens. Companion animals are also of zoonotic significance because of their close association with humans. In addition, they may be used as sentinels to monitor the distribution of ticks and the tick-borne pathogens they carry (Claerebout et al., 2013). There are estimated to be at least 7.62 million cats in the UK (pfma.org.uk, 2016), and the close proximity of humans and their pets highlights the importance of the 'One Health' approach to disease management (Day, 2011). The potential emergence of acaricide resistance and treatment deficiency in ticks and drug resistance in pathogens, leading to increased disease prevalence, cannot be ignored (Coles & Dryden, 2014). Furthermore, the increased movement of people and pets (van der Weijden et al., 2007), have increased the potential for the introduction and establishment of several novel vector species not previously present in some areas, together with the novel pathogens they may carry (Hartemink & Takken, 2016).

Many studies have investigated the prevalence of tick-borne pathogens in dogs in Great Britain (Smith *et al.*, 2011; Abdullah *et al.*, 2016, 2017), but ticks feeding on cats have recieved relatively less attention (Ogden et al., 2000), although the number of European studies has risen recently (Claerebout et al., 2013; Eichenberger et al., 2015; Pennisi et al., 2015; Krol *et al.*, 2016; Perischetti *et al.*, 2016). The interaction between cats and other potential hosts and their owners

is of particular interest, and is epidemiologically different to that of dogs. Cats have a wider, free-roaming behaviour and hunt, which brings them into contact with a greater range of diverse habitats and animals than dogs; cat grooming behaviour is also known to reduce the number of ectoparasites such as ticks and fleas (Ekstein & Hart, 2000).

A better understanding of the epidemiology of tick-borne pathogens and effective control of disease in cats requires a comprehensive knowledge of the key tick vectors and the pathogens they transmit (Wall, 2007). Unfortunately, our understanding of these factors is, in many cases, limited (Hill et al., 2005). The aim of the current investigation was to examine the distribution and species composition of ticks infesting cats in Great Britain and to determine the prevalence of two zoonotic pathogens, *Babesia* spp. and *Borrelia burgdorferi* sensu lato in the ticks collected.

2. Materials and Methods

2. 1 Tick collection and questionnaire

Following a nationwide publicity campaign to recruit veterinary practices, 278 participated between May and October 2016. Practices were sent a kit, consisting of an inspection protocol, envelopes, sample tubes and tick removers. The protocol instructed veterinary practitioners to select 5 cats per week at random from those vising the surgery for routine appointments, such as vaccination and general health checks, inspect them for ticks and complete a questionnaire describing the cat's clinical history (following the protocol described by Abdullah et al., 2016). The randomisation procedure to be adopted was not specified, however, veterinarians were asked not to choose cats for examination from geriatric/obesity clinics, since such cats may be disproportionately less likely to be exposed to tick infested habitats. A questionnaire for each animal was to be completed regardless of whether or not ticks were found, to allow tick prevalence to be calculated. Information requested included owner address, cat breed, sex, neutered status, presence and abundance of ticks, whether the cat had been abroad in the previous two weeks and its acaricidal treatment history. Veterinarians could print and mail the questionnaires or submit online. All tick samples were sent to the University of Bristol and stored at -20°C.

2.2. Data handling, statistical analysis and tick identification

Data was entered into a Microsoft Excel spreadsheet. For statistical analysis, age, sex, and hair-length were categorised as follows: <1, 1-3, 4-6, 7-10 and >10 years-of-age; female/male/neutered female/neutered male; longhaired or shorthaired. The WGS84 (World Geodetic System) map coordinates of each cat owner's location was recorded and classified as

urban or rural according to the UK Government's Output Area Population Weighted Centroids with the aid of the 'geosphere' package in R (R-Studio, version 1.0.136). The geographical program QGIS (Version 2.18.2) was used to map the location of samples. Statistical analysis was carried out in SPSS (Version 23). Binary logistic regression was used to identify associations between cat characteristics and the probability of carrying a tick.

Ticks were identified to species using a range of keys (Hillyard, 1996; Walker, 2003). Tick sex and life-cycle stage were noted. Female ticks were classified by level of engorgement as: unfed, partially-fed, or fully-fed. Fully-fed ticks were those considered to have reached maximum engorgement in relation to scutal dimensions; partially-fed ticks were defined as those that contained some blood but had not reached maximum expansion; unfed ticks contained no blood. The most developed and engorged tick per submission was selected for analysis. Tick infestation in relation to habitat and tick species were compared using chi-square analysis.

2.3 DNA extraction

After identification, ticks were cut transversely and longitudinally before carrying out DNA extraction on individual ticks using a Nucleospin ® 96 Tissue Core Kit (Macherey-Nagel, Germany) according to the manufacturer's guidelines. Preliminary trials showed that for fully-fed ticks, which contained large volumes of clotted blood, using the whole tick was not practical because after overnight digestion in double the recommended volume of Proteinase-K and tissue lysis buffer, the lysate clogged the silica column. To overcome this problem only the anterior two-thirds of the fully engorged tick (containing salivary glands) was used for DNA extraction and the extraction protocol used: 40 µl of Proteinase-K (instead of 30 µl) and 400 µl of tissue lysis buffer (instead of 240 µl), all samples were incubated at 56 °C overnight. After overnight digestion, only half of the lysate was used (using the full lysate volume still clogged the silica columns). An internal amplification control was introduced at this stage to check the efficacy of the DNA extraction in a PCR test prior to diagnostic PCR. Two repeats of wash buffer BW were used for each column prior to a single repeat of wash buffer B5, drying and elution in 100 µl of BE elution buffer. Spectrophotometry (Nanodrop) and agarose gel electrophoresis were used to estimate the DNA concentrations. DNA samples were stored in 96-well plates at -20°C until further analysis.

2.4 Babesia qPCR and sequence analysis

Babesia spp. were detected in DNA extracts using a probe based generic *Babesia* qPCR targeting the 18S rRNA gene. The following primers were used for detection of *Babesia* spp.: Babesia 944 for (5'-TTAACGAACGAGACCTTAACCTG-3'), Babesia 1315 rev (5'-

CCGAATAATTCACCGGATCAC-3') and Babesia TaqMan probe (5'-FAM-CGATCGGTAGGAGCGACGGGC-BHQ1-3') (Diagnostic Laboratories, Langford Vets, UK). A primer/probe mix was made as follows: 10 µM Babesia 944 for, 10 µM Babesia 1315 rev, 2.5 µM Babesia TaqMan probe. Positive (*Babesia canis*, 12763 gDNA diluted at 10^{-1}) and negative (water) controls were included in each 96 well PCR plate. The qPCR reaction was made with 2 µl of sample DNA and 8 µl of master mix, composed of 5 µl of 2x GoTaq Hot Start mix, 0.4 µl primer/probe mix, 0.6 µl 50 mM $MgCl_2$ and 2 µl H_2O. Thermal cycling conditions included an initial denaturation (95°C for 2 min; 45 cycles of 95 °C for 15 s, and 60 °C for 30 s) (Agilent MX3005P qPCR, Agilent, UK). Fluorescence data were collected at 520 nm at the end of each annealing/extension step. A cut off of over 35 cycles was used to differentiate true *Babesia* spp. positives from possible cross-reaction (see discussion). Samples positive on the *Babesia* spp. qPCR were re-amplified in a 25 µl volume for DNA sequencing.

DNA samples for sequence analysis were prepared using a Nucleospin® 96 PCR Clean-up Core Kit (Macherey-Nagel, Germany), before being sent to a commercial sequencing laboratory DNA Sequencing & Services (MRC I PPU, School of Life Sciences, University of Dundee, Scotland) using Applied Biosystems Big-Dye Ver 3.1 chemistry on an Applied Biosystems model 3730 automated capillary DNA sequencer. Resulting sequence data were analysed and tidied in BioEdit Sequence Alignment Editor (Version 7.2.5). The output from BioEdit was used to BLAST the NCBI GenBank sequence database (www.ncbi.nlm.nih.gov/BLAST/). Any sequences with less than 97% homology were not considered (Abdullah *et al.*, 2017).

2.5 Borrelia PCR and Sequencing

Conventional PCR was used to detect *B. burgdorferi* s.l. in the DNA extract; primers BSLF (5'-AATAGGTCTAATAATAGCCTTAATAGC-3') and BSLR (5'-CTAGTGTTTTGCCATCTTCTTTGAAAA-3') amplified a 250-300 bp region of the *ospA* gene found in all *B. burgdorferi* s.l. (Smith *et al*., 2012). Master mix was formulated as follows: 5 µl of 2x GoTaq Hot start mix (Promega, UK), 0.4 µl of 12.5 µM each BSLF/BSLR primer mix and 2.6 µl water. Two µl of extracted DNA were then added to 8 µL of master mix in 96 well PCR plates using a high throughput automated pipetting system (epMotion P5073, Eppendorf, UK). *Borrelia burgdorferi* sensu stricto (PCR product diluted $1x10^{-10}$) and water were used as positive and negative controls, respectively. The thermal cycling protocol consisted of an initial denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 20 s, 56 °C for 30 s and 72 °C for 30 s. Agarose gel electrophoresis was used to visualise target amplicons. Positive samples were identified as having a defined band of 250-300 bp on the gel and were later re-amplified in a 25 µl PCR for DNA sequencing as described above.

178

179 **3. Results**

180 *3.1 Tick species abundance and prevalence*

181 A total of 1,855 cats were inspected by 278 participating veterinary practices over a
182 period of 6 months between May and October 2016. These were broadly distributed
183 throughout Great Britain (Fig. 1). Six hundred and one cats were reported to have one or more
184 attached ticks. Due to damage some samples were unidentifiable, leaving 541 identified tick
185 samples from as many cats available for species identification and molecular analysis.

186 The tick species identified were *Ixodes ricinus* (57.1 %, n=309), *Ixodes hexagonus* (41.4
187 %, n=224), and *Ixodes trianguliceps* (1.5 %, n=8). Ticks were primarily adults (81.5 %, n=441)
188 and of these the majority were female (99.1 %, n=437) with only 4 males identified; these were
189 all *I. ricinus. Ixodes trianguliceps* were only found as nymphs. Partially-fed ticks were most
190 frequently recorded (68.9 %, n=373), followed by fully-fed ticks (22.6 %, n=122), and unfed
191 ticks (8.5 %, n=46). Twelve cats carried only tick larvae. On cats found to be carrying ticks, the
192 median intensity of infection was 1 with a maximum intensity of 84.

193 One of the aims of the study was to obtain a prevalence estimate for cat-tick infestation
194 and this was done by asking veterinary practices to examine a random selection of cats.
195 However, many of the veterinary practice staff appeared to not follow the inspection protocol
196 and submitted predominantly positive samples or submitted insufficient numbers of returns
197 per week. To determine a true prevalence for ticks on cats, practices that only submitted
198 questionnaires from cats with ticks and practices that submitted fewer than 5 returns per week
199 were removed. In this way, 1,127 cats from 248 veterinary practices were discounted, leaving
200 728 cats from 30 practices. Amongst these, 624 had access to the outdoors and of these 583
201 cats were negative and 41 were positive for ticks, giving an overall prevalence 6.6% (95%
202 confidence interval±1.94%). It is notable that the 30 practices that adhered strictly to the
203 protocol were responsible for almost 40% of the questionnaire submissions.

204

205 *2.2 Tick infestation risk-factors*

206 Binary logistic regression showed that age had a significant influence on the likelihood
207 of cats having ticks ($P < 0.05$); 4–6 year-old cats were the most likely to carry ticks ($P < 0.005$,
208 Exp(B) = 1.75, CI (95%) = 1.25-2.46), with cats below 1 and over 10 years of age being least
209 likely to carry ticks. Although the majority of cats included in the study were neutered, entire
210 cats were statistically more likely to be parasitized by ticks than neutered cats ($P < 0.001$,
211 Exp(B) = 2.33, CI (95%) = 1.68-3.23). For the analysis of hair length, mixed breed cats or cats of
212 no specified breed, where hair length could not be determined from the details provided, were

not included in the analysis (n=60). There was no significant influence of hair length on the probability of a cat carrying ticks ($P > 0.05$).

There were 1,565 cat owners who participated in the study, and of these 399 stated that their cats had preventative treatment for ticks. Although 464 cats were said to be treated with acaricide, only 95 cats (treated by 83 owners) had current protection based on the date of application and the product's specified label claim for residual activity. The remaining 369 cats had no active treatment against ticks; 229 cats (186 owners) had acaricidal treatment that was not active, and the remaining 140 cats (130 owners) were not treated with products that had an acaricidal label claim despite the owner's belief that their cats were protected against ticks. Almost one third (29%) of the treatment products reported by the owners had no acaricidal label claim. Of the 95 cats that did have current protection, 25 were found to be carrying ticks. There was no significant effect of preventative acaricide treatment on the probability of tick infestation ($P > 0.05$). Only one cat had been abroad within the 2 weeks before sampling and this animal had no ticks.

Cats classified as living in rural areas had a higher prevalence of ticks than cats living in urban areas ($P < 0.05$, Exp(B) = 1.34, CI (95%) = 1.07-1.67). For cats that were parasitized by ticks in large urban areas, *I. hexagonus* was most frequently recorded tick species (75.4 %, χ^2= 16, n= 43, $P < 0.001$). Both *I. ricinus* and *I. hexagonus* had a wide distribution throughout Great Britain, whilst *I. trianguliceps* was predominantly found in south eastern areas (Fig. 2).

2.3 Pathogen distribution

The internal amplification control was successfully amplified in all samples following qPCR. Of the 541 ticks that were analysed, 2.8% (n= 15) carried at least one *Babesia* spp. or *Borrelia burgdorferi* s.l pathogen. One tick contained a coinfection of both *Borrelia* spp. and *Babesia* spp. Pathogen DNA was found in *I. ricinus* and *I. hexagonus* ticks, but not in *I. trianguliceps* ticks. There were 59 potential positive tick samples for *Babesia* spp. after carrying out the initial qPCR assay. After DNA sequencing the qPCR positive PCR products and BLAST analysis, 1.1% (n=6) of these 59 tick samples were confirmed positive for *Babesia* spp. (95% confidence interval ±0.87%) (Table 1). Of these, four were *Babesia vulpes* sp. nov./*B. microti*-like and two were *Babesia venatorum*. The *Babesia vulpes* sp. nov./*B. microti*-like were all found in *I. hexagonus* ticks and the *B. venatorum* were only present in *I. ricinus.* Ticks containing *B. venatorum* were partially-fed adult females whereas *B. vulpes* sp. nov./*B. microti*-like were found in partially and fully-fed adults along with partially fed nymphs.

Initial *Borrelia burgdorferi* s.l. PCR indicated that 18 samples were positive, however, after DNA sequencing eight samples were removed because they gave non-target matches. The prevalence of *B. burgdorferi* s.l. was therefore 1.8% (95% confidence interval ±1.12%). These

included six *B. garinii* and four *B. afzelii* (Table 2). The majority of *B. burgdorferi* s.l. positives were found in partially-fed *I. ricinus* ticks. One unfed female contained *B. garinii* and one partially-fed *I. hexagonus* nymph was positive for *B. garinii.* One co-infection was identified in a partially fed *I. ricinus* female containing *B. venatorum* and *B. afzelii.*

Cases of *B. burgdorferi* s.l. were widely dispersed throughout Great Britain whereas *Babesia* spp. appeared to be more localised in the south (Fig. 3); sample sizes were too small to allow for meaningful statistical comparisons of pathogen species between habitat types.

4. Discussion

The most prevalent tick species found on cats was *I. ricinus*, which agrees with previous studies showing that this species is the most common tick in Europe (Beichel et al., 1996; Nijhof et al., 2007; Claerebout et al. 2013). However, *I. hexagonus* was also identified on a large number of cats; the prevalence of this tick on cats was considerably higher than has been reported previously on dogs. In the present study 41.4% of ticks were *I. hexagonus* whereas this species represented only 9.8% of the ticks found on dogs by Abdullah et al., (2016). Ogden et al. (2000) also found higher numbers of *I. hexagonus* on cats than dogs, but in that study *I. hexagonus* on cats was also more prevalent than *I. ricinus*. No differences between the prevalence of *I. hexagonus* on cats or dogs were observed in Germany (Beichel et al., 1996), The Netherlands (Nijhof et al., 2007) or Belgium (Claerebout et al. 2013). A higher prevalence of *I. hexagonus* on cats than dogs might be expected due to behavioural differences; cats actively hunt rodents, birds and amphibians (Churcher & Lawton, 1987) bringing them into contact with the habitat of the primary host of *I. hexagonus*, the common European hedgehog (*Erinaceus europaeus*) (Wierzbowska et al., 2016). In the present study, *I. hexagonus*, was most prevalent on cats in urban areas where populations of hedgehogs are known to be up to nine times higher than in forests, open grassland and agricultural land or rural areas (Young et al., 2006; Huijser et al., 1999; Hubert et al., 2011). In urban environments, *I. hexagonus* may therefore play an important epidemiological role in the transmission of pathogens, as suggested by Ogden et al. (2000) and Jahfari et al. (2017). The rodent tick, *Ixodes trianguliceps*, has not been reported previously on cats in Great Britain, but has been found on cats in Switzerland, but not dogs (Eichenberger et al., 2015).

The data suggest that 6.6% of cats in Great Britain with access to outside the home had ticks in the period between May and October 2016. Male, entire cats aged between 4 and 6 years living in rural areas were most likely to be infested. This may be due to variations in behaviour, with younger cats more likely to be active hunters and males having increased hunting success (Churcher & Lawton, 1987). Coat length had no significant effect on the probability of a cat having a tick. This could be the result of a genuine difference in tick attachment or represent the

difficulty of finding a tick on a long-haired cat during inspection. Acaricidal treatment also had no apparent effect in preventing tick attachment, but it was notable that 29% of the products listed by owners as being used for tick prevention had no acaricidal label claim. Owner recall may also have contributed to the very high number of cats with apparently expired tick treatments. This also highlights the need for veterinarians to ensure their pet owners know what the treatment prescribed protects against as well as the importance of educating on retreatment intervals.

The prevalence of *Babesia* spp. and *B. burgdorferi* s.l. in British cat ticks was relatively low: there were 6 cases of *Babesia* spp. (1.1%) and 10 cases of *B. burgdorferi* s.l. (1.8%). This is slightly lower than the 1.5% and 2.0% prevalences, respectively, recorded in ticks on dogs in Great Britain (Abdullah *et al.,* 2017). Conversely, studies identifying the pathogens present in *Ixodes* ticks infesting dogs and cats in Europe have reported prevalences of *Babesia* spp. of up to 9.0% in Poland (Krol *et al*., 2016), and 10.2% prevalence of *B. burgdorferi* s.l. in Belgium (Claerebout *et al.*, 2013). Nevertheless, similar to the data reported here on cats, Pennisi *et al* (2015) found a prevalence of *Babesia* spp. of 0.75% in Southern Italy. It must be noted however, that when pathogens are detected directly from the ticks rather than blood samples, the pathogen DNA may come either from ingested blood meal or represent a pre-existing infection, and these alternatives cannot be distinguished.

Babesiosis in domestic cats is relatively rare (Solano-Gallego & Baneth, 2011) and clinical signs of babesiosis in cats is thought not to occur in Europe. Clinical signs of infection with Lyme borreliosis in domestic cats is also extremely rare (Pantchev et al., 2016) in comparison to dogs. Analysis of cat sera in Portugal has found seroprevalences of *Babesia* spp. and *B. burgdorferi* s.l. of 6.6% and 2.2%, respectively (Maia et al., 2014). The lower rates of infection caused by *Babesia* spp. and *B. burgdorferi* s.l. in domestic cats in comparison to dogs may be the result of behavioural differences between cats and dogs, reduced awareness of signs of clinical infection or physiological and immunological differences in their response to infection (Day, 2016). Notably, *Babesia canis,* a common form of babesiosis in dogs, has only rarely been detected in cats (Solano-Gallego & Baneth, 2011).

Two *Babesia* spp. were identified here: *B. venatorum* and *B. vulpes* sp. nov./*B. microti*-like. These were identified in different tick species; *B. venatorum* was confined to *I. ricinus* and *B. vulpes* sp. nov./*B. microti*-like was confined to *I. hexagonus.* The latter also matched other *Babesia* piroplasms (Piroplasmida sp. mel1/Burgos/2007, *B. vulpes*, *Theileria annae* and *Babesia* cf. *microti*) sequences in the NCBI database with similar identity scores (Table 2) and it was difficult to assign them absolutely; Baneth et al. (2015) recently categorised them as a single species *B. vulpes* sp. nov, (Baneth et al., 2015), which was the approach adopted here.

Roe deer are the primary reservoir for *B. venatorum* (Najm *et al.*, 2014) and therefore its presence in *I. ricinus* is not unexpected. It has been suggested that *I. hexagonus* is the primary vector for *B. vulpes* sp. nov./*B. microti*-like (Camacho *et al.*, 2003), although this has been disputed (Najm *et al.*, 2014; Hodžić *et al.*, 2017), particularly since studies of engorged ticks could simply report the presence of pathogen DNA found in the host's blood and not necessarily tick-specific transmission (Hodžić *et al.*, 2017). Neither pathogen was identified in the *I. trianguliceps* samples, although numbers were very low. Previous studies conducted in the UK by Randolph (1991; 1995) found *I. trianguliceps* to be the principal vector for *B. microti*.

As previously shown in another European study (Rauter & Hartung, 2005), *B. afzelii* and *B. garinii* were the most common species of *Borrelia* detected in this study. *Borrelia garinii* and *B. afzelii* have been reported to circulate primarily through bird and rodent populations, respectively (Kurtenbach *et al.*, 2002).

The data presented in this study indicates that both *I. ricinus* and *I. hexagonus* are widely distributed in Great Britain, although the majority of *I. hexagonus* ticks were found in England, which has been noted previously (Abdullah et al., 2016) and *I. trianguliceps* was only found in south eastern England, supporting historical tick distribution records (Hubbard *et al.*, 1998). However, ticks from Wales and Scotland were not as well-represented as England in our study and so the tick-distribution maps are likely to have been affected by sample size bias. Too few *Babesia* spp. and *B. burgdorferi* s.l. were identified to allow meaningful statistical analysis of their spatial distribution or habitat differences.

The results presented here show that ticks can be found on cats throughout Great Britain and, although the prevalence may be relatively low, a range of species of *Babesia* and *B. burgdorferi* s.l. are present in these ticks. Cats may act as an important reproductive host for adult ticks, allowing maintenance of the tick population, and green spaces within urban areas are likely to form an important habitat for *I. hexagonus*, which is a known vector of pathogens (Jahfari et al., 2017).

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480

481 Table 1. The number, tick species, life-cycle stage, *Babesia* spp. identified on partial 18S rRNA
482 gene sequencing and sequence identity with matching GenBank accession numbers for the
483 analysed ticks.

484

Number of ticks	**Tick species**	**Tick life-cycle stage**	**Species detected**	**Sequence identity (%)**	**Accession Number**
2	*I. ricinus**	Partially fed adult	*B. venatorum*	99-100	KX008038
1	*I. hexagonus*	Fully fed adult	*B. vulpes sp. nov.*/*B. microti*-like	99	KT223483 KT580785 KJ871352 EU583387
1	*I. hexagonus*	Partially fed adult	*B. vulpes sp. nov.*/*B. microti*-like	99	KT223483 KT580785 KJ871352 EU583387
2	*I. hexagonus*	Partially fed nymph	*B. vulpes sp. nov.*/*B. microti*-like	99-100	KT223483 KT580785 KJ871352 EU583387

485 *One of these ticks had coinfection with *B. afzelii*
486

487

Table 2. The number, tick species, life-cycle stage and *Borrelia burgdorferi* s.l. species identified on partial *ospA* gene sequencing and sequence identity with matching GenBank accession numbers for the analysed ticks.

Number of ticks	**Tick species**	**Tick life-cycle stage**	**Species detected**	**Sequence identity (%)**	**Accession Number**
1	*I. hexagonus*	Partially fed nymph	*B. afzelii*	100	DQ007303
1	*I. ricinus*	Fully fed adult	*B. afzelii*	100	DQ007303
1	*I. ricinus*	Partially fed adult	*B. afzelii*	98	CP018263
1	*I. ricinus**	Partially fed adult	*B. afzelii*	100	DQ007300
1	*I. ricinus*	Partially fed adult	*B. garinii*	100	HM623293
1	*I. ricinus*	Partially fed adult	*B. garinii*	100	KU672587
1	*I. ricinus*	Partially fed adult	*B. garinii*	99	JF331369
1	*I. ricinus*	Partially fed adult	*B. garinii*	100	KU672587
1	*I. ricinus*	Partially fed adult	*B. garinii*	99	JF331361
1	*I. ricinus*	Unfed adult	*B. garinii*	98	KU051683

*Tick had coinfection with *B. venatorum*

506 **Figure legends**

507 Fig. 1. The distribution of veterinary practices participating in a survey of ticks on cats in Great
508 Britain.

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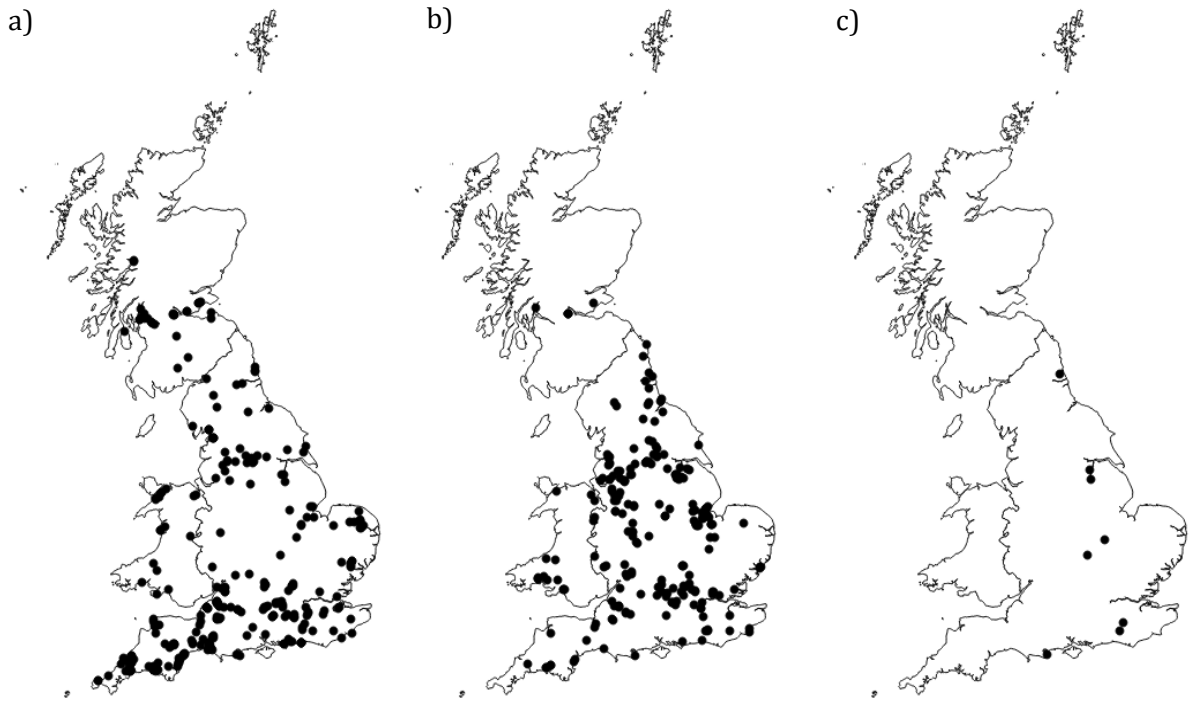
520 Fig. 2. The distribution of ixodid tick species found on cats in Great Britain: a) *Ixodes ricinus*, b)
521 *Ixodes hexagonus*, c) *Ixodes trianguliceps*



522

523 Fig. 3. The distribution of (a) *Borrelia burgdorferi* s.l. and (b) *Babesia* spp. in Great Britain. Open
524 shapes show *Babesia*: squares - *B. venatorum*, triangles - *B. vulpes* sp. nov./*B. microti*-like.
525 Solid shapes show *Borrelia*: circles - *B. garinii*, triangles - *B. afzelii* and star - *B. afzelii*-*B.*
526 *venatorum* co-infection

527 .