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# 1 Prevalence of ticks and tick-borne pathogens: *Babesia* and *Borrelia* 2 species in ticks infesting cats of Great Britain

3  
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## 10 11 **ABSTRACT**

12 In a study of tick and tick-borne pathogen prevalence, between May and October 2016, 278  
13 veterinary practices in Great Britain examined 1,855 cats. Six-hundred and one cats were found  
14 to have attached ticks. The most frequently recorded tick species was *Ixodes ricinus* (57.1%),  
15 followed by *Ixodes hexagonus* (41.4%) and *Ixodes trianguliceps* (1.5%). Male cats, 4 - 6 years of  
16 age living in rural areas were most likely to be carrying a tick; hair length and tick treatment  
17 history had no significant association with attachment. For cats that were parasitized by ticks in  
18 large urban areas, *I. hexagonus* was the most frequent species recorded. Molecular analysis was  
19 possible for 541 individual tick samples, others were too damaged for analysis; *Babesia* spp.,  
20 and *Borrelia burgdorferi* sensu lato were identified in 1.1% (n=6) and 1.8% (n=10) of these,  
21 respectively. *Babesia* spp. included *Babesia vulpes* sp. nov./*Babesia microti*-like (n=4) in *I.*  
22 *hexagonus* and *Babesia venatorum* (n=2) in *I. ricinus*. *Borrelia burgdorferi* s.l. species included  
23 *Borrelia garinii* (n=6) and *Borrelia afzelii* (n=4). The majority of *B. burgdorferi* s.l. cases were  
24 found in *I. ricinus*, with *B. afzelii* in one *I. hexagonus* nymph. No *Borrelia* or *Babesia* spp. were  
25 present in *I. trianguliceps*. To determine a true prevalence for ticks on cats, practices that only  
26 submitted questionnaires from cats with ticks and practices that submitted fewer than 5  
27 returns per week were removed; amongst those considered to have adhered strictly to the  
28 collection protocol, feline tick prevalence amongst cats that had access to the outdoors was  
29 6.6%. These results show that ticks can be found on cats throughout Great Britain, which  
30 harbour a range of species of *Babesia* and *B. burgdorferi* s.l. and that cats, particularly in green  
31 spaces within urban areas, may form an important host for *I. hexagonus*, a known vector of  
32 pathogens.

33  
34 *Key words: Ixodidae, Cat, Survey, Distribution, Zoonosis*

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## 37 **1. Introduction**

38 Ticks are an important group of arthropod vectors (Otranto & Wall, 2008) and transmit a  
39 wide range of viral, bacterial and protozoan pathogens. For three-host species in particular, their  
40 lack of host specificity allows them to feed on a different host in each life cycle stage and  
41 enhances their ability to transmit pathogens between host populations. In addition, their role as  
42 vectors is exacerbated by large population densities facilitated by high rates of reproduction, and  
43 the transmission of the pathogens they carry between the different life cycle-stages (trans-  
44 stadial transmission) while co-feeding and between generations via eggs (trans-ovarial  
45 transmission). Infection of the host with tick-transmitted pathogens may be aided by salivary  
46 anticoagulants and other active compounds that modulate host cutaneous immunity and  
47 inflammation, while enhancing vasodilatation to bring more blood to the feeding site (Wikel,  
48 1999). In the future, tick-borne disease may become an increasing concern as the changing  
49 climate favours tick survival in new geographical locations; previous studies have recorded  
50 correlations between tick population size and higher temperature (Lindgren et al., 2000; Gray et  
51 al., 2009; Tagliapietra et al., 2011; Jaenson et al., 2012; Korotkov et al., 2015). Associations  
52 between tick density and increasing host abundance have also been shown in multiple studies  
53 (Lindgren et al., 2000; Gilbert et al., 2012; Abdullah et al., 2016; Cat et al., 2017).

54 Companion animals are abundant, represent an easily available food source for ticks and  
55 may act as a reservoir for pathogens. Companion animals are also of zoonotic significance  
56 because of their close association with humans. In addition, they may be used as sentinels to  
57 monitor the distribution of ticks and the tick-borne pathogens they carry (Claerebout et al.,  
58 2013). There are estimated to be at least 7.62 million cats in the UK (pfma.org.uk, 2016), and the  
59 close proximity of humans and their pets highlights the importance of the 'One Health' approach  
60 to disease management (Day, 2011). The potential emergence of acaricide resistance and  
61 treatment deficiency in ticks and drug resistance in pathogens, leading to increased disease  
62 prevalence, cannot be ignored (Coles & Dryden, 2014). Furthermore, the increased movement of  
63 people and pets (van der Weijden et al., 2007), have increased the potential for the introduction  
64 and establishment of several novel vector species not previously present in some areas, together  
65 with the novel pathogens they may carry (Hartemink & Takken, 2016).

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

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

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

82

## 83 **2. Materials and Methods**

### 84 *2.1 Tick collection and questionnaire*

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

100

### 101 *2.2. Data handling, statistical analysis and tick identification*

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

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

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

119

### 120 *2.3 DNA extraction*

121 After identification, ticks were cut transversely and longitudinally before carrying out  
122 DNA extraction on individual ticks using a Nucleospin® 96 Tissue Core Kit (Macherey-Nagel,  
123 Germany) according to the manufacturer's guidelines. Preliminary trials showed that for fully-  
124 fed ticks, which contained large volumes of clotted blood, using the whole tick was not practical  
125 because after overnight digestion in double the recommended volume of Proteinase-K and  
126 tissue lysis buffer, the lysate clogged the silica column. To overcome this problem only the  
127 anterior two-thirds of the fully engorged tick (containing salivary glands) was used for DNA  
128 extraction and the extraction protocol used: 40 µl of Proteinase-K (instead of 30 µl) and 400 µl  
129 of tissue lysis buffer (instead of 240 µl), all samples were incubated at 56 °C overnight. After  
130 overnight digestion, only half of the lysate was used (using the full lysate volume still clogged  
131 the silica columns). An internal amplification control was introduced at this stage to check the  
132 efficacy of the DNA extraction in a PCR test prior to diagnostic PCR. Two repeats of wash buffer  
133 BW were used for each column prior to a single repeat of wash buffer B5, drying and elution in  
134 100 µl of BE elution buffer. Spectrophotometry (Nanodrop) and agarose gel electrophoresis  
135 were used to estimate the DNA concentrations. DNA samples were stored in 96-well plates at -  
136 20°C until further analysis.

137

### 138 *2.4 Babesia qPCR and sequence analysis*

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

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

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

163

#### 164 2.5 *Borrelia* PCR and Sequencing

165 Conventional PCR was used to detect *B. burgdorferi* s.l. in the DNA extract; primers BSLF  
166 (5'-AATAGGTCTAATAATAGCCTTAATAGC-3') and BSLR (5'-  
167 CTAGTGTTTTGCCATCTTCTTTGAAAA-3') amplified a 250-300 bp region of the *ospA* gene found  
168 in all *B. burgdorferi* s.l. (Smith *et al.*, 2012). Master mix was formulated as follows: 5 µl of 2x  
169 GoTaq Hot start mix (Promega, UK), 0.4 µl of 12.5 µM each BSLF/BSLR primer mix and 2.6 µl  
170 water. Two µl of extracted DNA were then added to 8 µL of master mix in 96 well PCR plates  
171 using a high throughput automated pipetting system (epMotion P5073, Eppendorf, UK).  
172 *Borrelia burgdorferi* sensu stricto (PCR product diluted 1x10<sup>-10</sup>) and water were used as positive  
173 and negative controls, respectively. The thermal cycling protocol consisted of an initial  
174 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  
175 for 30 s. Agarose gel electrophoresis was used to visualise target amplicons. Positive samples  
176 were identified as having a defined band of 250-300 bp on the gel and were later re-amplified in  
177 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  $\pm 1.94\%$ ). 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  $\text{Exp}(B) = 1.75$ ,  $\text{CI} (95\%) = 1.25\text{--}2.46$ ), 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  $\text{Exp}(B) = 2.33$ ,  $\text{CI} (95\%) = 1.68\text{--}3.23$ ). 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

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

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

227         Cats classified as living in rural areas had a higher prevalence of ticks than cats living in  
228 urban areas ( $P < 0.05$ ,  $\text{Exp}(B) = 1.34$ ,  $\text{CI} (95\%) = 1.07-1.67$ ). For cats that were parasitized by  
229 ticks in large urban areas, *I. hexagonus* was most frequently recorded tick species (75.4 %,  $\chi^2=$   
230 16,  $n= 43$ ,  $P < 0.001$ ). Both *I. ricinus* and *I. hexagonus* had a wide distribution throughout Great  
231 Britain, whilst *I. trianguliceps* was predominantly found in south eastern areas (Fig. 2).

232

### 233 2.3 Pathogen distribution

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

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



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

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

256

#### 257 **4. Discussion**

258 The most prevalent tick species found on cats was *I. ricinus*, which agrees with previous  
259 studies showing that this species is the most common tick in Europe (Beichel et al., 1996; Nijhof  
260 et al., 2007; Claerebout et al. 2013). However, *I. hexagonus* was also identified on a large number  
261 of cats; the prevalence of this tick on cats was considerably higher than has been reported  
262 previously on dogs. In the present study 41.4% of ticks were *I. hexagonus* whereas this species  
263 represented only 9.8% of the ticks found on dogs by Abdullah et al., (2016). Ogden et al. (2000)  
264 also found higher numbers of *I. hexagonus* on cats than dogs, but in that study *I. hexagonus* on  
265 cats was also more prevalent than *I. ricinus*. No differences between the prevalence of *I.*  
266 *hexagonus* on cats or dogs were observed in Germany (Beichel et al., 1996), The Netherlands  
267 (Nijhof et al., 2007) or Belgium (Claerebout et al. 2013). A higher prevalence of *I. hexagonus* on  
268 cats than dogs might be expected due to behavioural differences; cats actively hunt rodents,  
269 birds and amphibians (Churcher & Lawton, 1987) bringing them into contact with the habitat of  
270 the primary host of *I. hexagonus*, the common European hedgehog (*Erinaceus europaeus*)  
271 (Wierzbowska et al., 2016). In the present study, *I. hexagonus*, was most prevalent on cats in  
272 urban areas where populations of hedgehogs are known to be up to nine times higher than in  
273 forests, open grassland and agricultural land or rural areas (Young et al., 2006; Huijser et al.,  
274 1999; Hubert et al., 2011). In urban environments, *I. hexagonus* may therefore play an  
275 important epidemiological role in the transmission of pathogens, as suggested by Ogden et al.  
276 (2000) and Jahfari et al. (2017). The rodent tick, *Ixodes trianguliceps*, has not been reported  
277 previously on cats in Great Britain, but has been found on cats in Switzerland, but not dogs  
278 (Eichenberger et al., 2015).

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

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

292         The prevalence of *Babesia* spp. and *B. burgdorferi* s.l. in British cat ticks was relatively  
293 low: there were 6 cases of *Babesia* spp. (1.1%) and 10 cases of *B. burgdorferi* s.l. (1.8%). This is  
294 slightly lower than the 1.5% and 2.0% prevalences, respectively, recorded in ticks on dogs in  
295 Great Britain (Abdullah *et al.*, 2017). Conversely, studies identifying the pathogens present in  
296 *Ixodes* ticks infesting dogs and cats in Europe have reported prevalences of *Babesia* spp. of up to  
297 9.0% in Poland (Krol *et al.*, 2016), and 10.2% prevalence of *B. burgdorferi* s.l. in Belgium  
298 (Claerebout *et al.*, 2013). Nevertheless, similar to the data reported here on cats, Pennisi *et al*  
299 (2015) found a prevalence of *Babesia* spp. of 0.75% in Southern Italy. It must be noted however,  
300 that when pathogens are detected directly from the ticks rather than blood samples, the  
301 pathogen DNA may come either from ingested blood meal or represent a pre-existing infection,  
302 and these alternatives cannot be distinguished.

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

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

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

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

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

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

346

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355

356 **References**

- 357 Abdullah, S., Helps, C., Tasker, S., Newbury, H., Wall, R., 2016. Ticks infesting domestic dogs in  
358 the UK: a large-scale surveillance programme. *Parasit. Vectors.* **9**, 391.
- 359 Abdullah, S., Helps, C., Tasker, S., Newbury, H., Wall, R., 2017. Prevalence and distribution of  
360 *Borrelia* and *Babesia* in ticks feeding on dogs in the UK. *Med. Vet. Entomol* (In Press).
- 361 Baneth G, Florin-Christensen M, Cardoso L, Schnittger L. 2015. Reclassification of *Theileria*  
362 *annae* as *Babesia vulpes* sp. nov. *Parasit Vectors.* **8**, 207.
- 363 Beichel, E., Petney, T.N., Hassler, D., Brückner, M. and Maiwald, M., 1996. Tick infestation  
364 patterns and prevalence of *Borrelia burgdorferi* in ticks collected at a veterinary clinic in  
365 Germany. *Vet. Parasit.*, **65**, 147-155.
- 366 Camacho, A.T., Pallas, E., Gestal, J.J., Guitián, F.J., Olmeda, A.S., Telford, S.R., Spielman, A., 2003.  
367 *Ixodes hexagonus* is the main candidate as vector of *Theileria annae* in northwest  
368 Spain. *Vet. Parasitol.* **112**, 157-163.
- 369 Cat, J., Beugnet, F., Hoch, T., Jongejan, F., Prangé, A., Chalvet-Monfray, K., 2017. Influence of the  
370 spatial heterogeneity in tick abundance in the modeling of the seasonal activity of *Ixodes*  
371 *ricinus* nymphs in Western Europe. *Exp. Appl. Acarol.* **71**, 115-130.
- 372 Churcher, P.B. and Lawton, J.H., 1987. Predation by domestic cats in an English village. *J.*  
373 *Zool.* **212**, 439-455.
- 374 Claerebout, E., Losson, B., Cochez, C., Casaert, S., Dalemans, A.C., De Cat, A., Madder, M.,  
375 Saegerman, C., Heyman, P., Lempereur, L., 2013. Ticks and associated pathogens collected  
376 from dogs and cats in Belgium. *Parasit. Vectors.* **6**, 183.
- 377 Coles, T.B., Dryden, M.W., 2014. Insecticide/acaricide resistance in fleas and ticks infesting  
378 dogs and cats. *Parasit. Vectors.* **7**, 8.
- 379 Day, M.J., 2011. One health: the importance of companion animal vector-borne  
380 diseases. *Parasit. Vectors.* **4**, 49.
- 381 Day, M.J., 2016. Cats are not small dogs: is there an immunological explanation for why cats are  
382 less affected by arthropod-borne disease than dogs? *Parasit. Vectors.* **9**, 507.
- 383 Eichenberger, R.M., Deplazes, P., Mathis, A., 2015. Ticks on dogs and cats: A pet owner-based  
384 survey in a rural town in northeastern Switzerland. *Ticks. Tick. Borne. Dis.* **6**, 267-271.
- 385 Eckstein, R.A., Hart, B.L. 2000. Grooming and control of fleas in cats. *Appl. Anim. Behav. Sci.* **68**,  
386 141-150.
- 387 Gilbert, L., Maffey, G.L., Ramsay, S.L., Hester, A.J., 2012. The effect of deer management on the  
388 abundance of *Ixodes ricinus* in Scotland. *Ecol. Appl.* **22**, 658-667.
- 389 Gray, J.S., Dautel, H., Estrada-Peña, A., Kahl, O., Lindgren, E., 2009. Effects of climate change on  
390 ticks and tick-borne diseases in Europe. *Interdiscip Perspect Infect Dis.* **2009**, 1-12.

391 Hartemink, N., Takken, W., 2016. Trends in tick population dynamics and pathogen  
392 transmission in emerging tick-borne pathogens in Europe: an introduction. *Exp. Appl.*  
393 *Acarol.* **68**, 269-278.

394 Hill, C.A., Kafatos, F.C., Stansfield, S.K., Collins, F.H., 2005. Arthropod-borne diseases: vector  
395 control in the genomics era. *Nature Rev. Microbiol.* **3**, 262-268.

396 Hillyard, D., 1996. Ticks of north-west Europe. Field Studies Council. Field Studies Council  
397 Publications, Montford Bridge, U.K. 178 pp.

398 Hodžić, A., Zörer, J., Duscher, G.G., 2017. *Dermacentor reticulatus*, a putative vector of *Babesia cf.*  
399 *microti* (syn. *Theileria annae*) piroplasm. *Parasitol. Res.* **116**, 1075-1077.

400 Hubbard, M.J., Cann, K.J., Baker, A.S., 1998. Lyme borreliosis: a tick-borne spirochaetal  
401 disease. *Rev. Med. Microbiol.* **9**, 99-108.

402 Hubert, P., Julliard, R., Biagianti, S., Poulle, M.L., 2011. Ecological factors driving the higher  
403 hedgehog (*Erinaceus europeus*) density in an urban area compared to the adjacent rural  
404 area. *Landsc. Urban. Plan.* **103**, 34-43.

405 Huijser, M., Bergers, J., Ter Braak, C.J.F., 1999. The location of hedgehog traffic victims in  
406 relation to landscape features. *Lutra*, **42**, 57-58.

407 Jaenson, T.G., Jaenson, D.G., Eisen, L., Petersson, E., Lindgren, E., 2012. Changes in the  
408 geographical distribution and abundance of the tick *Ixodes ricinus* during the past 30  
409 years in Sweden. *Parasit. Vectors.* **5**, 8.

410 Jahfari, S., Ruyts, S.C., Frazer-Mendelewska, E., Jaarsma, R., Verheyen, K., Sprong, H., 2017.  
411 Melting pot of tick-borne zoonoses: the European hedgehog contributes to the  
412 maintenance of various tick-borne diseases in natural cycles urban and suburban  
413 areas. *Parasit. Vectors.* **10**, 134.

414 Korotkov, Y., Kozlova, T., Kozlovskaya, L., 2015. Observations on changes in abundance of  
415 questing *Ixodes ricinus*, castor bean tick, over a 35-year period in the eastern part of its  
416 range (Russia, Tula region). *Med. Vet. Entomol.* **29**, 129-136.

417 Król, N., Obiegala, A., Pfeffer, M., Lonc, E., Kiewra, D., 2016. Detection of selected pathogens in  
418 ticks collected from cats and dogs in the Wrocław Agglomeration, South-West Poland.  
419 *Parasit. Vectors.* **9**, 351.

420 Kurtenbach, K., De Michelis, S., Etti, S., Schäfer, S.M., Sewell, H.S., Brade, V., Kraiczy, P., 2002.  
421 Host association of the key role of host complement. *Trends. Microbiol.* **10**, 74-79.

422 Lindgren, E., Tälleklint, L., Polfeldt, T., 2000. Impact of climatic change on the northern latitude  
423 limit and population density of the disease-transmitting European tick *Ixodes*  
424 *ricinus*. *Environ. Health. Perspect.* **108**, 119.

425 Maia, C., Ramos, C., Coimbra, M., Bastos, F., Martins, Â., Pinto, P., Nunes, M., Vieira, M. L.,  
426 Cardoso, L., Campino, L., 2014. Bacterial and protozoal agents of feline vector-borne  
427 diseases in domestic and stray cats from southern Portugal. *Parasit. Vectors.* **7**, 115.

428 Najm, N.A., Meyer-Kayser, E., Hoffmann, L., Herb, I., Fensterer, V., Pfister, K., Silaghi, C., 2014. A  
429 molecular survey of *Babesia* spp. and *Theileria* spp. in red foxes (*Vulpes vulpes*) and their  
430 ticks from Thuringia, Germany. *Ticks. Tick. Borne. Dis.* **5**, 386-391.

431 Nijhof, A.M., Bodaan, C., Postigo, M., Nieuwenhuijs, H., Opsteegh, M., Franssen, L., Jebbink, F. and  
432 Jongejan, F., 2007. Ticks and associated pathogens collected from domestic animals in  
433 the Netherlands. *Vector. Borne Zoonotic Dis.*, **7**, 585-596.

434 Ogden, N.H., Cripps, P., Davison, C.C., Owen, G., Parry, J.M., Timms, B.J., Forbes, A.B., 2000. The  
435 ixodid tick species attaching to domestic dogs and cats in Great Britain and Ireland. *Med.*  
436 *Vet. Entomol.* **14**, 332-338.

437 Otranto, D., Wall, R., 2008. New strategies for the control of arthropod vectors of disease in  
438 dogs and cats. *Med. Vet. Entomol.* **22**, 291-302.

439 Pantchev, N., Vrhovec, M.G., Pluta, S., Straubinger, R.K., 2016. Seropositivity of *Borrelia*  
440 *burgdorferi* in a cohort of symptomatic cats from Europe based on a C6-peptide assay  
441 with discussion of implications in disease aetiology. *Berl. Munch. Tierarztl. Wochenschr.*  
442 **129**, 333.

443 Pennisi, M.G., Persichetti, M.F., Serrano, L., Altet, L., Reale, S., Gulotta, L., Solano-Gallego, L.,  
444 2015. Ticks and associated pathogens collected from cats in Sicily and Calabria (Italy).  
445 *Parasit. Vectors.* **8**, 512.

446 Perischetti, M.F., Solano-Gallego, L., Serrano, L., Altet, L., Reale, S., Masucci, M., Pennisi, M.G.,  
447 2016. Detection of vector-borne pathogens in cats and their ectoparasites in Southern  
448 Italy. *Parasit. Vectors.* **9**, 247.

449 Pfma.org.uk. (2016). *Cat Population Detail 2016*. Available at: [http://www.pfma.org.uk/cat-](http://www.pfma.org.uk/cat-population-2016)  
450 [population-2016](http://www.pfma.org.uk/cat-population-2016).

451 Randolph, S.E., 1991. The effect of *Babesia microti* on feeding and survival in its tick vector,  
452 *Ixodes trianguliceps*. *Parasitology.* **102**, 9-16.

453 Randolph, S.E., 1995. Quantifying parameters in the transmission of *Babesia microti* by the tick  
454 *Ixodes trianguliceps* amongst voles (*Clethrionomys glareolus*). *Parasitology.* **110**, 287-295.

455 Rauter, C., Hartung, T., 2005. Prevalence of *Borrelia burgdorferi* sensu lato genospecies in  
456 *Ixodes ricinus* ticks in Europe: a metaanalysis. *Appl. Environ. Microbiol.* **71**, 7203-7216.

457 Smith, F.D., Ballantyne, R., Morgan, E.R., Wall, R., 2011. Prevalence, distribution and risk  
458 associated with tick infestation of dogs in Great Britain. *Med. Vet. Entomol.* **25**, 377-384.

459 Smith, F.D., Ballantyne, R., Morgan, E.R., Wall, R., 2012. Estimating Lyme disease risk using pet  
460 dogs as sentinels. *Comp. Immunol. Microbiol. Infect. Dis.* **35**, 163-167.

461 Solano-Gallego, L., Baneth, G., 2011. Babesiosis in dogs and cats—expanding parasitological  
462 and clinical spectra. *Vet. Parasitol.* **181**, 48-60.

463 Tagliapietra, V., Rosà, R., Arnoldi, D. Cagnacci, F., Capelli, G., Montarsi, F., Hauffe, H.C., Rizzoli, A.,  
464 2011. Saturation deficit and deer density affect questing activity and local abundance of  
465 *Ixodes ricinus* (Acari, Ixodidae) in Italy. *Vet. Parasitol.* **183**, 114-124.

466 van der Weijden, W.J., Marcelis, R.A., Reinhold, W., 2007. 24. Invasions of vector-borne diseases  
467 driven by transportation and climate change. In: Takken, W., Knols, B.G. (Eds.), *Emerging*  
468 *pests and vector-borne diseases in Europe*. Wageningen Academic Pub. 439 pp.

469 Walker, A. (2003). *Ticks of domestic animals in Africa*. Edinburgh: Bioscience Reports, pp. 3-  
470 210.

471 Wall, R., 2007. Ectoparasites: future challenges in a changing world. *Vet. Parasitol.* **148**, 62-74.

472 Wierzbowska, I.A., Hędrzak, M., Popczyk, B., Okarma, H., Crooks, K.R., 2016. Predation of  
473 wildlife by free-ranging domestic dogs in Polish hunting grounds and potential  
474 competition with the grey wolf. *Biol. Conserv.* **201**, 1-9.

475 Wikel, S.K., 1999. Tick modulation of host immunity: an important factor in pathogen  
476 transmission. *Int. J. Parasitol.* **29**, 851-859.

477 Young, R., Davison, J., Trewby, I.D., Wilson, G.J., Delahay, R.J., Doncaster, C., 2006. Abundance of  
478 hedgehogs (*Erinaceus europaeus*) in relation to the density and distribution of badgers (*Meles*  
479 *meles*). *J. Zool.* **269**, 349-356.

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>I. ricinus</i> *	Partially fed adult	<i>B. venatorum</i>	99-100	KX008038
1	<i>I. hexagonus</i>	Fully fed adult	<i>B. vulpes sp. nov./B. microti-like</i>	99	KT223483 KT580785 KJ871352 EU583387
1	<i>I. hexagonus</i>	Partially fed adult	<i>B. vulpes sp. nov./B. microti-like</i>	99	KT223483 KT580785 KJ871352 EU583387
2	<i>I. hexagonus</i>	Partially fed nymph	<i>B. vulpes sp. nov./B. microti-like</i>	99-100	KT223483 KT580785 KJ871352 EU583387

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

486

487



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

491

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

492 \*Tick had coinfection with *B. venatorum*

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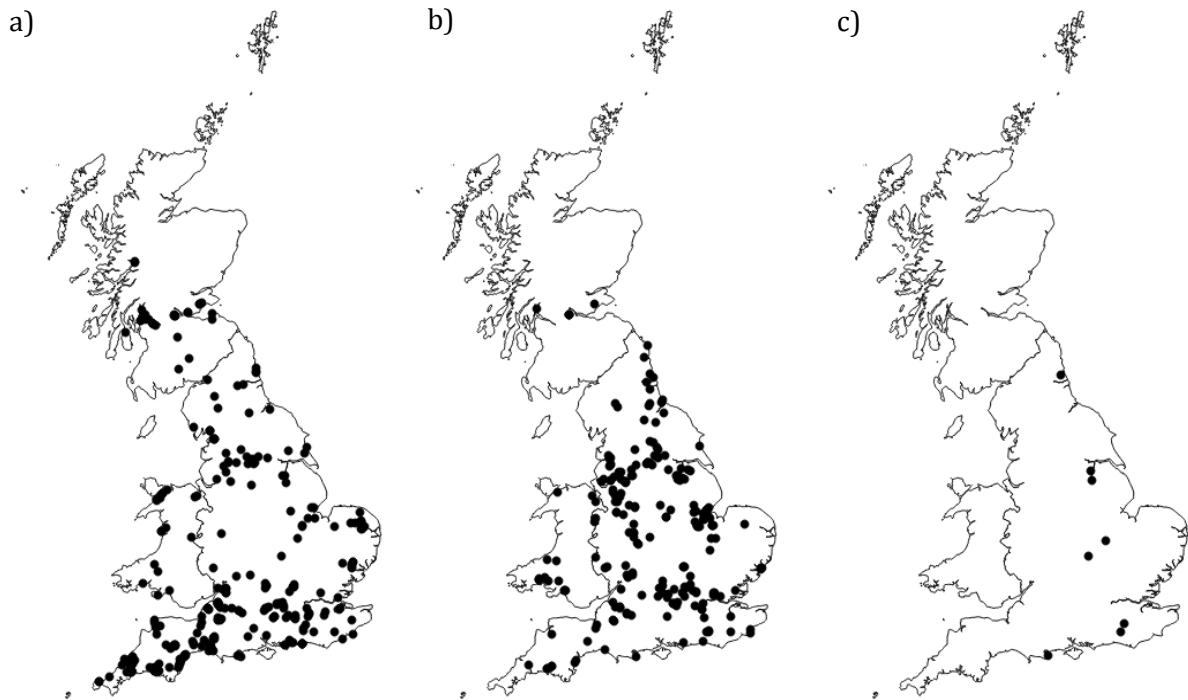
506 **Figure legends**

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