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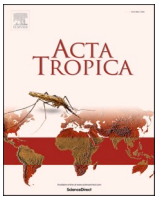

Prevalence of *Babesia* spp. pathogens in the ticks *Dermacentor reticulatus* and *Ixodes ricinus* in the UK

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ABSTRACT

The emergence of *Babesia* pathogens novel to the UK is of growing concern; these include *Babesia canis* and *Babesia caballi*. However, a better understanding of changes in the prevalence of endemic *Babesia* species such as *Babesia venatorum* and *Babesia divergens* is also of importance. Here, the prevalence of *Babesia* pathogens in both *Dermacentor reticulatus* and *Ixodes ricinus* ticks was assessed. *Dermacentor reticulatus* were collected from six sites known to harbour populations of this species in west Wales and southern England. DNA was extracted from 879 individual ticks and subjected to PCR and sequence analysis. Seven *Babesia* species were detected in 7.5% of the ticks, including *B. caballi* (0.68%), *B. bovis* (1.7%), *B. microti* (1.02%), *B. bigemina* (0.34%), *B. capreoli* (0.34%), and one isolate of *B. canis* (0.34%). Two of the field sites with grazing equines present had ticks that were positive for *B. caballi*. For *I. ricinus*, up to 200 nymphs were collected from each of 24 cattle farms in south-west England. Nymphs were divided into 6 pools of 30 from each farm for DNA extraction, PCR, and sequencing. Samples from seven out of the 24 farms tested positive for *Babesia*, and most were positive for more than one species. *Babesia divergens* was identified from five farms, and three of these farms had two pooled samples positive for *B. divergens*, which given the low overall prevalence rate suggests that *B. divergens* may be highly clustered within the tick population. Most of the remaining positive samples were *Babesia venatorum*, demonstrating that this zoonotic pathogen is widespread in livestock habitats. The data suggest that *B. canis* is not yet widely prevalent in established *D. reticulatus* populations in the UK, but that there is a need to raise awareness of the risk of equine piroplasmosis in areas with endemic *D. reticulatus* foci, since *B. caballi* appears more widely established.

1. Introduction

Babesia spp. are protozoan haemoparasites transmitted by ticks, with a near ubiquitous global presence (Schnittger et al., 2012, 2022). They are the second most commonly found parasite in the blood of mammals after trypanosomes, and of significant economic, veterinary and medical concern. *Babesia* is the causative agent of the disease babesiosis (piroplasmosis), the severity of which varies with *Babesia* species and the immune status of the animal, but ranges from mild illness to acute disease, severe haemolysis and eventually death (Solano-Gallego and Baneth, 2011). Ticks acquire *Babesia* infections by feeding on an infected host, but transovarial transmission of *Babesia canis* has been observed through up to five tick generations (Chauvin et al., 2009). Humans usually only become susceptible to babesiosis if splenectomised or otherwise immunocompromised, and *B. divergens* or *B. microti*, parasites of cattle and rodents respectively, have been indicated as the most common causal agents (Gray et al., 2010; Mørch et al., 2015).

Recent decades have seen an increase in the reported prevalence of *Babesia* spp. in ticks and cases of babesiosis in animals in Europe (Matjila et al., 2005; Beugnet and Marié, 2009). For endemic *Babesia*, these increases may be associated with changes in the distribution and abundance of both the tick vectors and hosts. For novel *Babesia* species, the threat of the introduction and establishment may be associated with climate change and greater dispersal of people and animals. For example, in the UK, although currently not considered to be of major concern, equine piroplasmosis caused by *Babesia caballi* is a significant emerging threat (Coultous et al., 2019). The low prevalence of endemic equine piroplasmosis in the UK has previously been attributed to the

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geographical limitations of its vector tick *Dermacentor reticulatus*, however expansion of the range of this tick, as seen in central and northern Europe (Nadal et al., 2021) may be a risk factor for future higher transmission (Sands et al., 2021). Acute cases present with anaemia, pyrexia, lethargy, dehydration and anorexia, which may result in death (Schnittger et al., 2022). However, chronic and sub-clinical cases can produce a latent carrier state. Importation of these carrier horses to different regions of the UK may be a route by which *B. caballi* could become more widely established (Coultous et al., 2019).

Five *Babesia* species are known to affect dogs: *B. canis, B. vogeli, B. rossi, B. gibsoni* and *B. vulpes sp. nov.* (Matijatko et al., 2012; Baneth et al., 2015; Ciuca et al., 2021), the most pathogenic and widespread being *B. canis*, which is endemic in most of continental Europe and transmitted by *D. reticulatus* (Criado-Fornelio et al., 2000; Halos et al., 2013, 2014; Bajer et al., 2022). In the UK, there has been an increasing number of cases of babesiosis in imported dogs (Shaw et al., 2003), and *B. canis* has been found in a *D. reticulatus* tick collected from a dog that had recently travelled into the UK from Europe (Abdullah et al., 2018). The first case of fatal babesiosis in a dog that had not left the UK was diagnosed in Kent and the likely causal agent was tentatively identified as *B. vogeli* (Holm et al., 2006). Subsequently a cluster of cases of *B. canis* was reported involving dogs in Essex with no history of overseas travel (Hansford et al., 2016; de Marco et al., 2017; MacLeod and Wright, 2019), suggesting that *B. canis* might have become endemic in this area. The distribution of *B. canis* is closely associated with its vector *D. reticulatus* and therefore changes in the distribution of this tick is an important risk factor (Wright, 2018). Historical records show that *D. reticulatus* has been found in isolated populations in the UK for over 100 years and at least four established, predominantly coastal, populations have been confirmed (Jameson and Medlock, 2011; Sands et al., 2021).

Ixodes ricinus is the primary vector of *B. divergens* and *B. major*, and these are the *Babesia* pathogens that principally affect cattle in the UK. Bovine babesiosis can cause significant morbidity and mortality in cattle (Johnson et al., 2020) and south-west England is known to have a particularly high prevalence (Lihou et al., 2020). In infected cattle intraerythrocytic *Babesia* merozites cause lysis of erythrocytes in the process of asexual division, leading to haemoglobinaemia, haemoglobinuria and pyrexia (Christensson, 1989; Sherlock et al., 2000). It is the haemoglobinuria which gives babesiosis the name redwater. Clinical cases are generally seen when naïve cattle are exposed to infected pastures as adults (McFadzean et al., 2021) and, in these naïve adult hosts, infection may cause death within a few days (Collins et al., 1970). Milder forms of the disease, associated with juvenile or immune hosts, are characterized by pyrexia and anorexia for a period of several days (Christensson, 1989). Higher seroprevalence of *B. divergens* in cattle herds have been associated with farms with higher tick densities in France (Agoulon et al., 2012). While bovine babesiosis has long been endemic in the UK, changes in the distribution and abundance of *I. ricinus* may potentially be associated with increased disease risk.

The aim of this study, therefore, was to investigate the prevalence of *Babesia* spp. in both *D. reticulatus* and *I. ricinus* in the UK, to confirm the current prevalence of endemic species such as *B. divergens* in their vector populations, but particularly to establish whether novel potential threats to the UK, such as *B. canis* or *B. caballi*, could be detected.

2. Material and methods

2.1. Tick collection

Sampling of *D. reticulatus* was carried out between 2019 and 2020, at six sites in the UK where there are known populations of *D. reticulatus* (Medlock et al., 2017). Sites included two coastal dune systems in Gwynedd, Wales; Morfa Harlech and 8 miles south, Morfa Dyffryn. These contained some small bodies of water and were grazed by cattle. Collection sites on the north coast of Devon were at Braunton Burrows, a dune area of approximately 1400 hectares grazed by cattle, and Northam Burrows Country Park, a saltmarsh and dune system grazed by horses and sheep. On the south coast of Devon, Bolt Tail headland immediately to the southwest of Hope Cove was sampled, and was grazed by sheep. Finally, a site in Essex was sampled; Old Hall Marshes, an RSPB nature reserve containing many bodies of water, extensive saltmarsh, reedbeds and marshes grazed by cattle and horses. Ticks were collected by blanket dragging (n=656) where a white cloth sheet (1 m × 1 m) was dragged slowly across the vegetation and turned over every 5 m, and ticks attached to the sheet were then removed and collected. *Dermacentor reticulatus*, collected by Public Health England between the years of 2015–2020 (n=216), and ticks sent from veterinary professionals during the study (n=7), from sites in Essex, Wales and Devon were also used for the analysis. Of the ticks collected by PHE, 24 were collected from dogs and 192 from the environment. Two of the ticks sent from veterinary professionals were collected from dogs and five from ponies.

Twenty-six farms in south-west England were visited between March and June 2021 to collect ticks from cattle grazing pastures. The farms were selected based on the results of a longitudinal questionnaire survey reported elsewhere (Lihou et al., 2020), in which farmers had reported ticks infesting their cattle, and from vet practices and farm organisations in the south-west. Farms did not have to have experienced tick-borne disease in their livestock to be recruited to the study. The farm grazing areas visited consisted of a mix of improved, semi-improved and rough pastures, including moorland. The ticks were collected by blanket dragging using a white cotton sheet. The aim was to collect up to 200 nymphs per farm, to allow the detection of prevalence as low as 1.5% at a confidence level of 95% in the tick population (Win Episcope v2.0; Thrusfield et al., 2001). A minimum of 30 drags were conducted per farm, to ensure sample collection was not clustered in the environment. The number of drags and the number of ticks per drag were counted. Drags were never repeated over the same location more than twice. In rough grazing pastures, drags were not limited to edge habitats, and at least one drag length was left in-between each drag, to increase the area surveyed.

For *I. ricinus,* pooled samples were used and the number of positive pools, the total number of pools, and the pool size were used to estimate the overall prevalence of *Babesia* spp., using an online pooled prevalence calculator (Epitools online prevalence calculator; Sergeant, 2018). This method assumed a fixed pool size and a perfect test sensitivity and specificity. Exact confidence limits were calculated based on binomial theory, so that confidence limits were between 0 and 1. Pooled sampling is common in population studies of infectious agents in vectors such as ticks, where low prevalence rates mean that large sample sizes are required, and more negative results are expected than positive results (Abel et al., 1999; Furstenau et al., 2020). Pooling samples may increase the likelihood of false negatives by reducing test sensitivity, however pools of 30 ticks were considered acceptable in the present study, given the sample size of 5097 *I. ricinus* nymphs (Furstenau et al., 2020). Pools of up to 39 have been found to maintain test accuracy for n=1536 samples (Furstenau et al., 2020) with minimum pool size increasing with n.

2.2. Tick identification, DNA extraction, PCR and sequence analysis

Ticks were identified based on morphological characteristics (Estrada-Peña et al., 2017). Ticks were stored in ethanol at -20 °C pending DNA extraction. Each tick was cut longitudinally and transversely before DNA extraction, which was performed using a commercially available extraction kit (DNeasy Blood & Tissue Kit, Qiagen GmbH, Hilden, Germany). For *I. ricinus,* ticks from each farm were divided at random into 6 pools of 30 nymphs for DNA extraction, PCR, and sequencing (N=129). Ticks were digested using 20 μL of Proteinase-K and 180 μL of tissue lysis buffer, incubated at 56 °C in a thermal mixer for three hours. After digestion the resulting lysate was transferred to spin columns and processed following the manufacturer's

instructions. Finally, DNA was eluted in 80 μL of elution buffer and stored at − 20 °C prior to further 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 forward (5′-TTAACGAACGAGACCTTAACCTG-3′), Babesia 1315 reverse (5′-CCGAATAATTCACCGGATCAC-3′) and Babesia TaqMan probe (5′-FAM-CGATCGGTAGGAGCGACGGGC-BHQ1–3′) (Diagnostic Laboratories, Langford Vets, Bristol, U.K.). A primer/probe mix was made with 10 μm *Babesia* 944 forward, 10 μm Babesia 1315 reverse and 2.5 μm Babesia TaqMan probe. Positive (*B. canis*, 52,835 PCR product diluted at 10−2) 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 MgCl2 and 2 μL of water. Thermal cycling conditions were 95 °C for 2 min; 45 cycles of 95 °C for 15 s, and 60 °C for 30 s (Agilent MX3005P qPCR; Agilent Technologies UK Ltd, Edinburgh, U.K.). Fluorescence data were collected at 520 nm at the end of each annealing/extension step. Positive PCR samples were later re-amplified in a 25-μL PCR reaction for DNA sequencing.

Amplicons were prepared for DNA sequencing (NucleoSpin® 96 PCR Clean-up Core Kit; Macherey-Nagel GmbH & Co. KG) and sent for commercial DNA sequencing [Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC PPU) DNA Sequencing and Services, School of Life Sciences, University of Dundee, Dundee, U.K.] using Applied Biosystems Big Dye Version 3.1 chemistry on a model 3730 automated capillary DNA sequencer (Applied Biosystems, Inc., Foster City, CA, U.S.A.). Sequences were checked and edited, if necessary, using BioEdit Sequence Alignment Editor Version 7.2.5 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), or Unipro UGENE (v41.0; Okonechnikov et al., 2012) and then compared with sequence data available in GenBank using blastn (http://www.ncbi.nlm.nih.gov/BLAST/). The 'top hit' was taken as the sequence with the highest query cover and percentage identity cover. Any sequences with percentage homology of less than 90–95% or an E value of <0.05 were not considered. For *I. ricinus*, because the individual samples were pooled, 'secondary hits' were also considered from other *Babesia* spp. if they had a query cover and percentage identity of greater than 98%.

3. Results

Only adult *D. reticulatus* were found by blanket dragging. DNA was extracted from 879 individuals and subjected to PCR and, where a positive PCR result was detected, subsequent sequence analysis was undertaken. Due to the high sensitivity of the PCR which was designed to detect *Babesia* spp. in dog blood, cross-reactivity occurred with other tick-borne microorganisms. This issue has been reported previously for *Babesia* detection in ticks (Abdullah et al., 2018) and does not occur with DNA from dog blood where other endocelluar symbionts are not found. Therefore, DNA sequence analysis was necessary to avoid false positive results, and a subset of 294 ticks were sequenced. The analysis showed a prevalence of infection by *Babesia* of 7.5% (22/294). Seven *Babesia* species were detected (Table 1; Fig. 1) including *B. canis* (0.34%; n=1), *B. caballi* (0.68%; n=2), *B. bovis* (1.7%; n=5), *B. microti* (1.02%; n=3), *B. bigemina* (0.34%; n=1), *B. capreoli* (0.34%; n=1); the remainder (3.06%; n=9) could not be classified unambiguously to species. All positive samples were from ticks that were collected from the environment, except one of the ticks positive for *B. caballi* which was collected from a pony.

Ixodes ricinus was found on all of the 26 farms visited in s.w. England; however adequate sample sizes were only collected from 24 farms. No other tick species were found in the samples collected at these sites. A total of 5097 nymphs were collected. After PCR and sequencing, 10 (7.8%; ±4.6; N=129) pooled samples were found to be positive for *Babesia* spp. Pools positive for *Babesia* were identified from seven (29.2%; ±18.2; N=24) different farms (Fig. 1). Three different species of *Babesia* were identified by nucleotide BLAST search (Table 2). *Babesia divergens* was identified in eight (6.2% ±4.2; N=129) pooled samples and on five (20.8% ±16.3; N=24) farms. The estimated prevalence of *Babesia* spp. in the sampled tick population was 0.27% (95% exact C.I: 0.13 – 0.46; Sergeant, 2018). The estimated prevalence of *B. divergens*, based on the number of pools where *B. divergens* was identified, was 0.21% (95% exact C.I: 0.091 – 0.42). All the remaining positive samples were identified as *B. venatorum* or *B. capreoli* (Table 2).

Table 1
Babesia species identified in *Dermacentor reticulatus* collected from sites of known populations in the UK, sequence identity and E()-value with matching GenBank accession numbers for the analysed ticks.

Site	*Babesia* species	Sequence identity (%)	E ()-value	Accession number
Morfa Harlech/ Morfa Dyffryn	*Babesia bovis*	96.08	2E-12	MN124163.1
	Babesia capreoli	93.24	2E-20	MG344771.1
	Babesia sp.	93.75	2E-16	AF411338.1
	Babesia sp.	93.44	9E-15	AF411338.1
Braunton Burrows	*Babesia microti/ bigemina*	91.8	4E-13	MH208613.1 (microti) /KY805829.1 (bigemina)
	Babesia sp.	91.87	5E-38	KT895089.1
	Babesia sp.	93.94	2E-17	AF411338.1
Northam Burrows	*Babesia canis*	97.22	3E-24	MK571833.1
	Babesia bovis	92.14	2E-29	LC169076.2
	Babesia microti	94.12	2E-18	MH208611.1
	Babesia microti	93.65	9E-16	MH208611.1
	Babesia caballi	95.92	3E-11	JQ417260.1
	Babesia sp.	93.44	8E-15	AF411338.1
Bolt Tail	*Babesia microti*	94.67	1E-22	MH208611.1
	Babesia sp.	94.34	2E-36	MF162312.1
	Babesia sp.	91.22	3E-47	KT895089.1
Old Hall Marshes	*Babesia caballi*	100	0.0007	KX349898.1
	Babesia bovis	92.37	6E-38	MH045765.1
	Babesia bovis	100	0.00009	MH045765.1
	Babesia bovis	100	0.0001	MH045765.1
	Babesia sp.	93.94	1E-32	MF162312.1
	Babesia sp. 1	95	2E-25	JX984667.1

4. Discussion

Increases in prevalence and distribution of tick-borne disease has been reported in various parts of Europe in recent years (Beugnet and Marié, 2009). Such changes are the result of a complex interaction of factors, particularly increases in tick abundance and distribution driven by climatic factors allied to changes in land-use, habitat management and wild host abundance. Changes in tick-borne pathogen prevalence is of particular concern in the UK, since it sits at the northern edge of the range of one of the key vectors, *D. reticulatus* – a tick that has seen rapid expansion of its range in mainland Europe (Drehmann et al., 2020).

The outbreak of canine babesiosis in the UK highlighted the need for a better understanding of the prevalence and risk from emerging *Babesia* pathogens (Hansford et al., 2016) since *B. canis* had not previously been detected in an established field population and was entirely confined to animals that had travelled to continental Europe (Smith et al., 2013).

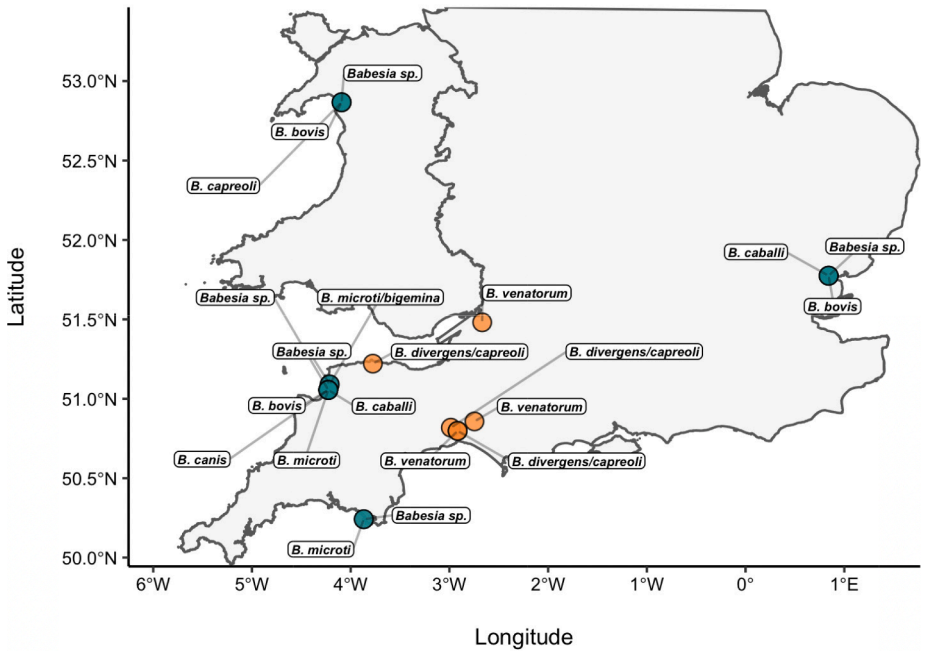

Fig. 1. Map of *Babesia* pathogens detected in *Ixodes ricinus* ticks collected from farms in south-west England (orange points) and *Dermacentor reticulatus* ticks collected from known UK populations (blue points).

Here, *B. canis* was found in a single adult *D. reticulatus* tick collected by blanket dragging in North Devon, in south-west England. Only adult *D. reticulatus* were analysed because no larvae or nymphs were ever collected in the blanket drags. This does suggest that the pathogen may be present at low prevalence more widely than previously thought. The report of what appeared to be *B. gibsoni*-like pathogens in the UK in the study by Smith et al. (2013) was likely to have been due to methodological flaws and, in the absence of supporting evidence, should probably be discounted.

A recent survey of the European prevalence and seroprevalence of *B. caballi*, the causative agent of equine piroplasmosis, gave an estimated seroprevalence of 8% and prevalence of 2% (Nadal et al., 2021). However, historically *B. caballi* has not been considered problematic in the UK and this has been attributed largely to the patchy distribution of its vector *D. reticulatus* (Sands et al., 2021). Evaluation of the prevalence of equine piroplasmosis by both serology and PCR in 1242 equine samples in the UK gave a serological prevalence of 8% with parasite DNA found in 0.8% of samples (Coultous et al., 2019). Of particular interest in the current study, therefore, was that *B. caballi* was detected in *D. reticulatus* from two sites in Essex and north Devon. Both sites where ticks infected with *B. caballi* were found had grazing ponies, and one of these ticks was collected directly from a pony. It should be noted that the detection of tick-borne pathogens may be higher in feeding than in questing ticks (Reye et al., 2012). Since no vaccine is available against equine piroplasmosis and screening is not currently required for equine importation into the UK (Coultous et al., 2019), greater awareness and improved preventive measures are essential for this pathogen. In this study *B. bovis, B. bigemina, B. microti, B. capreoli* were also detected in *D. reticulatus.* These cause babesiosis in cattle, rodents and deer respectively, and *B.bovis* was found in north Wales, Essex and north Devon. This highlights the risk posed, should *D. reticulatus* expand its distribution from its current largely coastal populations (Sands et al., 2021).

Babesia divergens is found across northern Europe, largely following the distribution of *I. ricinus* (Zintl et al., 2003). Here, for *I. ricinus* collected from cattle pastures in south-west England, *B. divergens* was detected at five of the 24 farms surveyed, with three farms having two pooled samples that were positive for *B. divergens*; given the low overall prevalence rate, this suggests that this pathogen may be highly clustered within the tick population. This is supported by highly aggregated positive samples recorded from specific pastures on a beef farm in Germany (Springer et al., 2020). A prevalence of 3 and 4% of *I. ricinus* positive for *B. divergens* was reported from one study in Lithuania and Norway, respectively, with prevalence higher in adults, and females (Radzijevskaja et al., 2008). However, most studies investigating tick populations in northern European report overall prevalences of about 1% across life cycle stages (Øines et al., 2012; Hamšíková et al., 2016; Davies et al., 2017; Abdullah et al., 2018). Here, only nymphal *I. ricinus* were analysed, because inadequate numbers of adults could be obtained. The estimated pathogen prevalence in the sampled tick population was 0.2% for *B. divergens*, however the true prevalence of *Babesia* may well be higher than recorded here as the sample sizes used in the study were designed to detect prevalences as low as 1.7% and therefore some false negatives were likely. Taylor et al. (1982) found regional farm prevalences of 64–93%, with cattle testing positive for *B. divergens* antibodies throughout Northern Ireland. However, there is evidence that Redwater cases have been declining in Ireland in recent years, although due to warming winters, cases can now occur at any time of year (Zintl et al., 2014). Reports of Redwater as early as February have also now been reported in the UK (Johnson et al., 2020).

Babesia venatorum is a widespread zoonotic parasite largely associated with wild deer. In a survey of 4750 *I. ricinus* ticks collected from dogs in the UK, seventy were positive for *Babesia* spp.: 84.3% were *Babesia venatorum* (Abdullah et al., 2018). A molecular examination of *Babesia* infecting sheep in the United Kingdom found sequences positive for *B. venatorum* (Gray, 2019). Here, *B. venatorum* was also the most abundant *Babesia* spp. found in *I. ricinus* collected from cattle pastures, demonstrating that this parasite is widespread in livestock, raising concerns for public health and disease management. Babesia species were distributed similarly on farm sites across the sampling area in south-west England, with *B. venatorum, B. divergens* and *B. capreoli* found on farms from both the north and the south of the region (Fig. 1). This also suggests that these *Babesia* species largely follow the distribution of *I. ricinus* in south-west England.

In conclusion, the emergence of novel *Babesia* species and the changes in distribution and prevalence of endemic *Babesia* species,

Table 2
Babesia species detected in *Ixodes ricinus* ticks from pooled samples, collected from cattle pasture at 24 farms in southwest England: sequence identity and E ()-value with matching GenBank accession numbers for the analysed ticks.

Farm number (pool)	*Babesia* species	Sequence identity (%)	E() value	Accession number
1 (1)	*B. venatorum*	96.12%	1.00E-113	KU204799.1
14 (1)	*B. divergens / capreoli*	100.00%	8.00E-141	MG344781.1 / MG344782.1
14 (1)	*B. venatorum*	98.56%	4.00E-134	KX008038.1
16 (1)	*B. venatorum*	100.00%	1.00E-93	MG344777.1
16 (1)	*B. divergens*	98.44%	1.00E-88	MG344781.1
16(1)	*B. capreoli*	98.44%	1.00E-88	MG344779.1
16(2)	*B. venatorum*	100.00%	7.00E-147	MG344777.1
16(2)	*B. divergens*	98.61%	3.00E-140	AY098643.2
16(2)	*B. capreoli*	98.26%	1.00E-138	MG344782.1
17 (1)	*B. divergens / capreoli*	100.00%	3.00E-57	MG344781.1 / MG344782.1
21(1)	*B. venatorum*	98.78%	5.00E-118	MG344777.1
24(1)	*B. divergens / capreoli*	100.00%	9.00E-146	MG344781.1 / MG344782.1
24(1)	*B. venatorum*	98.60%	4.00E-139	KX008038.1
24(2)	*B. venatorum*	100.00%	3.00E-73	MG344777.1
24(2)	*B. capreoli*	98.06%	3.00E-68	MG344782.1
24(2)	*B. divergens*	98.06%	3.00E-68	MG344781.1
25(1)	*B. venatorum*	100.00%	7.00E-111	MG344777.1
25(1)	*B. capreoli*	98.65%	7.00E-106	MG344782.1
25(1)	*B. divergens*	98.65%	7.00E-106	MG344781.1
25(2)	*B. venatorum*	100.00%	2.00E-146	MG344777.1
25(2)	*B. divergens*	98.61%	1.00E-139	AY098643.2
25(2)	*B. capreoli*	100%	5.00E-138	MG344782.1

resulting from changes in tick abundance, climate, landscape use and population movements mean that a greater awareness of and surveillance for these pathogens is essential to allow timely and meaningful interventions.

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CRediT authorship contribution statement

Bryony Sands: Data curation, Methodology, Writing – original draft. **Katie Lihou:** Methodology, Data curation, Writing – review & editing. **Philippa Lait:** Supervision, Formal analysis. **Richard Wall:** Conceptualization, Supervision, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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