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Original article

Limited occurrence of *Borrelia burgdorferi* sensu lato in the European hedgehog (*Erinaceus europaeus*) and *Ixodes hexagonus* in Great Britain

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ABSTRACT

Borrelia burgdorferi sensu lato (*Bbsl*) are multi-host bacteria and the causative agents of the zoonotic disease, Lyme borreliosis, for which *Ixodes* spp. are the vectors. In Great Britain (GB), research to date has primarily focussed on *Ixodes ricinus* as the main tick transmitting this pathogen, while the role that the European hedgehog (*Erinaceus europaeus*) and the hedgehog tick (*Ixodes hexagonus*) might have in the transmission cycle requires investigation. This study aimed to examine the occurrence of *Bbsl* infection in hedgehogs and *I. hexagonus* in GB; to characterise the species if present; and to better inform our understanding of these species as potential hosts or vectors. Post-mortem examinations have been conducted on hedgehogs found dead from across GB over the period 2013–2022 inclusive. We collated the available convenience sample archive from 96 hedgehogs for which both frozen ear tissue and *Ixodes* spp. (comprising 563 *I. hexagonus*, 18 *I. ricinus* and one *Ixodes frontalis*) in 70 % ethanol were available. Supplementary tissue samples were analysed from the hedgehogs where either ear tissue or ticks tested *Borrelia* DNA-positive, to investigate whether the infection was localised or disseminated. An additional 86 *I. hexagonus* collected from 14 hedgehogs with no ear tissue available were included to increase the sample size. DNA from tissue and tick samples was tested using a pan-*Borrelia* qPCR assay. Only 4.2 % (4/96) of hedgehogs and 1.2 % (4/335 total: 0.6 %, 2/329 *I. hexagonus*; 40 %, 2/5 *I. ricinus*) of tick pools were qPCR-positive suggesting that *Bbsl* infrequently circulate in hedgehog and *I. hexagonus* in GB. Therefore, both species may play a limited role in wider transmission cycles in this country. *Borrelia afzelii* was the sole species characterised by subsequent sequence analysis in both hedgehogs and ticks, providing some evidence of host-vector interaction at larval and nymph life stages, as all the positive ticks were collected from *B. afzelii* DNA-positive hedgehogs. Histopathological examination of hedgehog tissues found no evidence of borreliosis and therefore no clinical significance of *B. afzelii* infection to hedgehog health. The low occurrence of *B. afzelii* detected in *I. hexagonus,* combined with the lower frequency of human biting behaviour of *I. hexagonus* when compared with *I. ricinus,* suggests that the public health risk of infection from *I. hexagonus* bites is lower than for *I. ricinus.* Notably, our dataset found minimal co-feeding of these tick species on hedgehog hosts in contrast to studies in mainland Europe, which could influence pathogen dynamics in GB.

1. Introduction

Multi-host pathogens require a comprehensive understanding of community dynamics to evaluate their transmission networks and maintenance within a system (Buhnerkempe et al., 2015). In the case of *Borrelia burgdorferi* sensu lato (*Bbsl*) species complex, the causative agents of the zoonotic disease, Lyme borreliosis, the spirochaetal bacteria are transmitted by hard ticks of the *Ixodes* genus (Buhnerkempe et al., 2015). *Ixodes ricinus,* the main *Bbsl* vector, parasitises a variety of hosts and may transmit the pathogen to mammals or birds (Mannelli et al., 2012). *Ixodes hexagonus* feeds primarily on the European hedgehog (*Erinaceus europaeus*, hereafter hedgehog), and less frequently parasitises different species, including other wildlife, domestic animals and humans (Gern and Humair, 2002; Gern et al., 1991; Ogden et al., 2000;

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Walker, 2018). To date the majority of published studies have focused on *I. ricinus*, and the potential role of *I. hexagonus* in the maintenance and circulation of the pathogen remains poorly understood (Gray and Kahl, 2022).

Borrelia species distribution varies depending on the occurrence of competent tick vectors and reservoir host(s) (Margos, 2019). *Borrelia afzelii, B. garinii* and *B. burgdorferi* sensu stricto are the three main pathogenic species found in Europe, which are maintained by key vertebrate species (Gray and Kahl, 2022). *Borrelia afzelii* circulates mostly in rodent populations, such as wood mice (*Apodemus sylvaticus*) and bank vole (*Myodes glareolus*) (Hanincova et al., 2003); while *B. garinii* is associated with birds, for example blackbirds (*Turdus merula*) and song thrushes (*Turdus philomelos*) (Humair et al., 1998; Michalik et al., 2008; Taragel'ová et al., 2008). In contrast, *B. burgdorferi* sensu stricto can be found in various species (Ytrehus and Vikøren, 2012), including various rodents and squirrels (*Sciurus* spp.).

In Great Britain (GB), studies on *Ixodes* spp. found on various wildlife species have detected *Borrelia* spp. including *B. afzelii* in *I. ricinus* collected from red squirrels (*Sciurus vulgaris*) (Luu et al., 2021) and grey squirrels (*Sciurus carolinensis*) (Millins et al., 2015), while multiple *Bb*sl species were detected in an *I. hexagonus* dataset limited to 57 ticks collected from Eurasian badgers (*Meles meles*) and hedgehogs in a rehabilitation centre in South West England (Couper et al., 2010). Otherwise, studies on *I. hexagonus* have focused on ticks collected from dogs and cats, of which only three adult females (out of 386 ticks) and one pool of nymphs (out of 224 ticks), respectively, were found to be infected, all with *B. afzelii* (Abdullah et al., 2018; Davies et al., 2017). In contrast, in mainland Europe, various investigations have detected *B. afzelii, B. bavariensis, B. garinii, B. burgdorferi* sensu stricto and *B. spielmanii* in *I. hexagonus* and hedgehogs, where both species appear to form part of the bacterium's transmission cycle (Jahfari et al., 2017; Krawczyk et al., 2015; Majerová et al., 2020; Skuballa et al., 2012; Terriere et al., 2022).

The conservation status of the European hedgehog has been recently classified as "near threatened" on the IUCN Red List, as the population has declined markedly in recent decades (Gazzard et al., 2025; Wembridge et al., 2022): whilst the drivers for this reduction are believed to be multifactorial, the potential contribution of disease requires further investigation. Hedgehogs inhabit a range of habitat types, including gardens, where supplementary food provision is increasingly common, and they are also the most frequently admitted wild mammal in rehabilitation centres in GB (Molony et al., 2006). This potential human-wildlife interface warrants investigation into the occurrence of zoonotic pathogens in hedgehogs and disease risk pathways relevant to public health (Krawczyk et al., 2015; Lawson et al., 2018; Sangster, 2016; Terriere et al., 2022). Furthermore, surveillance data show that humans in the UK are bitten by *I. hexagonus* (Hansford et al., 2023a), highlighting the importance of investigating potential public health impacts.

The aim of this study is to investigate the occurrence of *Borrelia* spp. infection in hedgehogs and *I. hexagonus*, to characterise the species if present, and to use this information to infer the potential role of both species in the transmission cycle of *Borrelia* species in GB.

2. Materials and methods

1. Sample collection

Samples were collected as part of a national general disease surveillance programme (www.gardenwildlifehealth.org) for the hedgehog which began in 2013. Members of the public report observations of hedgehog mortality from across GB leading to carcass submission for investigation of cause of death in a subset of incidents. Post-mortem examinations were conducted following a standardised protocol, comprising subjective assessment of the state of carcass preservation, systematic inspection of external and internal organs combined with routine parasitological and microbiological examinations, supported with further ancillary diagnostic testing as dictated based on macroscopic findings (Franklinos et al., 2015; Lawson et al., 2018). A suite of frozen tissue samples was archived as routine, including ear tissue at −80 °C. Tissue samples were also fixed in buffered 10 % formal saline pending histopathological examination, where permitted by the state of tissue preservation. Metazoan parasites including *Ixodes* spp. were collected and preserved in 70 % ethanol at room temperature.

Ear tissue was selected from the available convenience sample archive for detection and characterisation of *Borrelia* spp. since this has previously shown to be highly sensitive in detecting spirochetes (Sinsky and Piesman, 1989), and it yielded the highest proportion of positive detections (compared with other tissues such as urinary bladder, heart, liver) in hedgehogs (Majerová et al., 2020; Terriere et al., 2022).

For each hedgehog from which both ear tissue and ticks were included in this study, the habitat type where the animal was found was categorised as urban, suburban or rural classification provided by the Department for Environment Food & Rural Affairs (DEFRA 2016), and by reviewing the postcode areas in Google Maps (www.google.com/maps).

Additional ticks collected from hedgehogs at post-mortem examination for which no ear tissue samples were available, were also included in the study. All ticks were examined using a stereomicroscope for species identification based on morphological features (Estrada-Peña et al., 2017; Hillyard, 1996). Following identification, adult ticks were stored in individual tubes, while nymphs and larvae were grouped in pools of up to five and ten specimens respectively, for DNA extraction.

In the event of *Borrelia* spp. detection in either hedgehog ear tissue or *Ixodes* spp., supplementary tissues from the frozen archive were subsequently also screened by qPCR to investigate whether host infection, if present, was localised or disseminated. Urinary bladder, kidney, muscle and heart were selected for this purpose, since these were reported with greatest frequency of *Bb*sl detection in free-living wildlife after ear tissue (Majerová et al., 2020; Millins et al., 2015; Skuballa et al., 2012; Terriere et al., 2022).

2. *Borrelia* screening

DNA was extracted from hedgehog tissue samples using the QIAGEN DNAeasy Blood and Tissue kit® (Qiagen, Hilden, Germany) as per the manufacturers protocol, with the inclusion of two negative extraction controls (i.e., no-template control with reagents only and an amphibian tissue template control).

The same Qiagen kit was used for DNA extraction from ticks, with minor modification to the Qiagen's supplementary protocol for purification of DNA from ticks for detection of *Borrelia* DNA (QIAGEN 2008). Briefly, each sample was transferred into a Precellys tube with 180 µl buffer ATL + 20 µl proteinase K and homogenised using the Precellys 24 Homogenizer, programme 5 (Bertin technologies, Montigny-le-Bretonneux, France). The samples were mixed by vortexing, incubated in a thermomixer for $\geq$ 5 h and subsequently processed according to the manufacturer's instructions. DNA quality and concentration was appraised in a subset of tissue DNA extracts using the NanoDrop ND-1000® (Marshall Scientific, New Hampshire, USA).

A pan-*Borrelia* spp. qPCR assay targeting a 148 bp fragment of the 16S rRNA gene was used (adapted from Parola et al., 2011) in both hedgehog tissues and ticks. PCR reactions were prepared in a total volume of 20 µl, which comprised: 10 µl of ABI TaqMan Fast Universal master mix (Applied Biosystems, ThermoFisher Scientific, USA), 1 µl of primer/probe mix (0.18 µl forward primer: 16S forward 5′-AGC CTT TAA AGC TTC GCT TGT AG-3′; 0.18 µl reverse primer: 16S reverse 5′-GCC TCC CGT AGG AGT CTG G-3′; 0.05 µl probe: 5′−6FAM- CCG GCC TGA GAG GGT GAACGG-BHQ1 3′; 0.59 µl H_2O (PCR grade), 4 µl H_2O (PCR grade) and 5 µl template DNA. A QuantoStudio 7 Flex real-time PCR system (Applied Biosystems) was used with a hold step for 20 s at 95 °C, followed by 40 cycles of 3 s at 95 °C and 30 s at 60 °C.

To characterise the *Borrelia* species, qPCR-positive samples were further subjected to a conventional PCR targeting ~250 bp of the 5S-23S rRNA intergenic spacer region. Each PCR mix of 50 µl comprised: 5 µl 10 × PCR reaction buffer ($MgCl_2$), 1 µl 10 mM dNTPs, 1.5 µl 50 mM $MgCl_2$, 2 µl of each primer from a 10 µM stock (5S-23S forward 5′-GAG TTC GCG GGA GAG TAG GTT ATT GCC-3′, 5S-23S reverse 5′-TCA GGG TAC TTA GAT GGT TCA CTT CC-3′), 0.2 µl Platinum *Taq* DNA polymerase (Invitrogen, ThermoFisher Scientific, USA), 33.3 µl PCR grade H_2O and 5 µl of template DNA. A LabTech G-Storm Thermocycler was used with the following cycling conditions: 5 min at 94 °C, followed by 10 cycles of 94

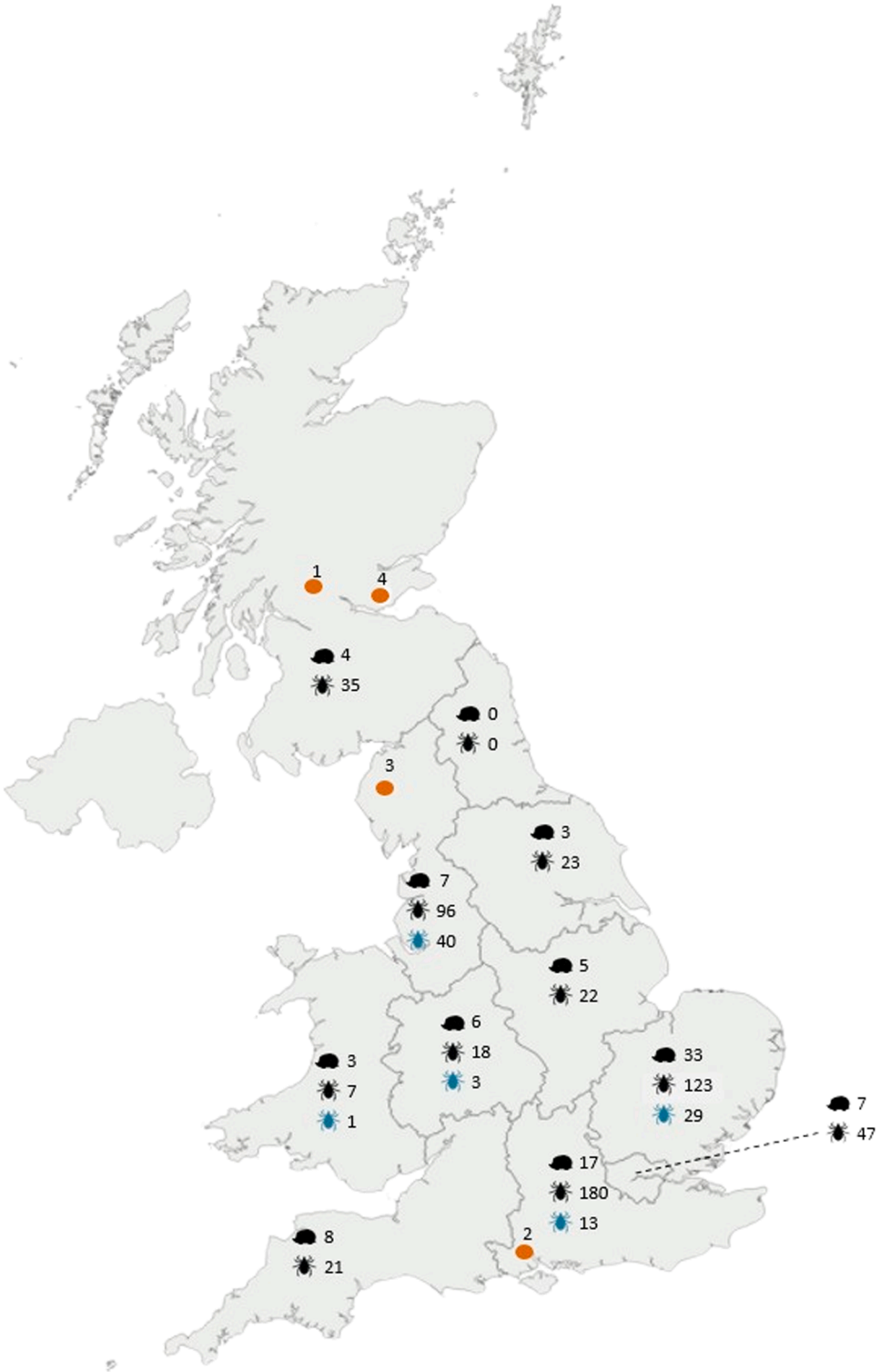

Fig. 1. European hedgehogs (*Erinaceus europaeus*) (🦔) and *Ixodes* ticks (🕷) collected from Scotland, Wales and England regions, 2013–2022 inclusive. The dotted line refers to the samples collected in Greater London. The figure includes 93 hedgehogs and 563 ticks (in black), excluding three hedgehogs and nine ticks for which location data were missing. In addition, 86 *I. hexagonus* ticks collected from hedgehogs for which ear tissue was not available are shown (in blue). The orange dots show the locations for the *Borrelia* qPCR-positive cases found in: Sterling (case 1, 2015) and Fife (case 4, 2019) in Scotland; Hampshire (case 2, 2015) and in Lancashire (case 3, 2015) in the South East and North West of England, respectively.

°C for 20 s, 70 °C for 30 s (lowering by 1 °C each cycle) and 72 °C for 30 s, then 40 cycles of 94 °C for 20 s, 60 °C for 30 s and 72 °C for 30 s, with a final extension of 72 °C for 7 mins (Hansford et al., 2023b). PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter, California, USA) according to the manufacturer's protocol, DNA concentrations measured using Qubit dsDNA high sensitivity assay kit (Applied Biosystems, ThermoFisher Scientific, USA) and subjected to bidirectional Sanger sequencing. The sequences were trimmed using SnapGene Viewer software (Dotmatics, Massachusetts, USA). Alignment and consensus sequences were obtained using MEGA11 software (Tamura et al., 2021), and then screened against the NCBI (National Centre for Biotechnology Information) BLAST database (http://www.ncbi.nlm.nih.gov/BLAST/).

3. Histological examination

Histological examination was conducted on tissues from the four hedgehogs that tested qPCR-positive on tissue and/or tick samples, to appraise if there was evidence of Lyme borreliosis as determined by inflammation in the presence of spirochetes. A suite of tissues from the formalin-fixed archive was selected from each case with particular focus on microscopic examination of the brain ($n = 3$), heart ($n = 4$), kidney ($n = 4$), liver ($n = 4$), and spleen ($n = 4$) where available, since spirochetes have been isolated in these tissues from a wild fox (V*ulpes vulpes schrencki)* (Isogai et al., 1994), and meningoencephalitis, nephritis and hepatitis have been reported with Lyme borreliosis in domestic and wild animals (Dambach et al., 1997; Isogai et al., 1994; Johnstone et al., 2016). While arthritis, dermatitis, conjunctivitis and uveitis have also been described in affected animals (Isogai et al., 1994; Johnstone et al., 2016), no formalin-fixed eyes, skin (excluding Case 3 where this tissue was examined) or joints were available from the hedgehogs investigated. Tissues were processed using standard methods (Supplementary Table 2). Each tissue was stained with Haematoxylin and Eosin (H&E), while kidney tissues were also examined using Masson's Trichrome and Periodic Acid Schiff histochemical stains to evaluate potential fibrosis and glomerular changes associated with borreliosis (Dambach et al. 1997), respectively. Additional histochemical stains, comprising Gram, Ziehl-Neelsen and Warthin-Starry silver, were used to further characterise potential pathogens noted on examination of H&E sections.

3. Results

The dataset included 668 ticks, of which 582 ticks were collected from 96 hedgehogs with available ear tissue (Fig. 1), and 86 ticks from a further 14 hedgehogs examined post-mortem from which ear tissue was not available for testing. The hedgehogs were found dead over the period 2013–2022 inclusive, with a distribution across GB although skewed towards southern England (Fig. 1). Habitat type was characterised as urban (222 ticks tested from 43 hedgehogs), suburban (132 ticks tested from 24 hedgehogs) and rural (209 ticks tested from 26 hedgehogs), with detailed location data missing from three hedgehogs.

In total, 649 *I. hexagonus* (190 adult females, four adult males, 400 nymphs and 55 larvae), 18 *I. ricinus* (two adult females, ten nymphs and six larvae) and one *I. frontalis* (adult female) were identified (Table 1). The average number of ticks tested was 6.1 per hedgehog (range 1–45). Ninety-three hedgehogs were infested with *I. hexagonus*. One hedgehog was infested with both *I. ricinus* and *I. hexagonus* (one female per species), while one hedgehog – found in Fife, Scotland –was infested only with *I. ricinus* (six larvae, ten nymphs and one adult female). An adult female *I. frontalis* was found co-infesting with *I. hexagonus* in one hedgehog.

The overall *Borrelia* spp. occurrence was 4.2 % (4/96) in hedgehogs, and 1.2 % (4/335) in tick samples. Positive hedgehog ear tissue was found in two out of 96 samples, while the other two tested positive in supplementary tissues tested (see below). Positive ticks comprised one pool of *I. hexagonus* larvae ($n = 3$), one pool of *I. hexagonus* nymphs ($n = 5$), one pool of *I. ricinus* larvae ($n = 6$) and one pool of *I. ricinus* nymphs ($n = 5$), collected from three hedgehogs. The positive *I. ricinus* larvae and nymph pools were from the same hedgehog. Two tick pools were from hedgehogs with positive ear tissues, while ear tissue tested negative from the remaining two animals: however, the bacteria were detected in supplementary tissues in both cases. Heart, kidney, muscle and urinary bladder tissues were positive for the hedgehog with positive *I. hexagonus* pool larvae, while heart and kidney tested positive for the hedgehog infested with positive *I. ricinus* larvae and nymphs. One hedgehog with positive ear tissue had positive *I. hexagonus* nymphs, while all ticks tested negative from the second animal (45 ticks divided in 14 pools: four females, 39 nymphs and two larvae) (Table 2).

Table 1
Total number (no.) and number of *Borrelia*-positive (pos.) *Ixodes* tick species collected from European hedgehogs (*Erinaceus europaeus*) by life stage. The table shows the number of pooled ticks for the pan-*Borrelia* qPCR assay.

Tick Species	Life stage			
	Adult (Female)	Adult (Male)	Nymphs	Larvae
	No.	No.	**pos.**/pool[a] (No.)	**pos.**/pool[a] (No.)
I. hexagonus	190	4	**1**/123 (400)	**1**/12 (55)
I. ricinus	2	-	**1**/2 (10)	**1**/1 (6)
I. frontalis	1	-	-	-

[a] max 5 nymphs/pool
[b]max 10 larvae/pool.

The two *Borrelia*-positive hedgehogs with infected *I. hexagonus* nymphs and larvae were found in a sub-urban area of Stirling in Scotland in September 2015 and in a rural area of Hampshire in South East England in October 2015, respectively. The positive hedgehog with no infected ticks was collected from a sub-urban area in Lancashire in North West England in October 2015, while the positive hedgehog infested with positive *I. ricinus* larvae and nymphs was collected from a rural habitat in Fife, Scotland, in September 2019.

Sequence analysis confirmed the species as *B. afzelii* for ten qPCR-positive samples (246 – 273 bp) with 100 % identity to *B. afzelii* in the NCBI database (Genbank Accession No OP882493). Two samples could not be typed despite repeated sequencing attempts: one ear tissue sample from a hedgehog with no associated infected ticks and qPCR-negative supplementary tissues; and one urinary bladder sample with all other supplementary tissues and *I. hexagonus* larvae positive and confirmed by sequencing.

No evidence of spirochetes nor significant pathological findings consistent with borreliosis were detected on microscopic examination of tissues of *Borrelia*-qPCR positive hedgehogs; with very minimal, non-specific changes of heart (Case 2 and 4) and kidney (Case 1, 2 and 4) tissues observed (Supplementary Tables 1 and 2). The causes of death in these cases were diagnosed as verminous pneumonia ($n = 2$), salmonellosis ($n = 1$) and trauma ($n = 1$).

4. Discussion

In this study, a low occurrence of *Bb*sl in hedgehogs and *I. hexagonus* ticks was detected in GB, through screening of an available convenience sample. This contrasts with the findings of a similar previous study where investigation of a range of tissues (i.e., heart, kidney, liver, lung, spleen, urinary bladder) collected from hedgehogs ($n = 32$) that died in rehabilitation centres, and *I. hexagonus* (number not reported) collected from these hosts, failed to detect the bacteria (Skuballa et al., 2012). In comparable studies conducted in various countries in mainland Europe, *Borrelia* infection has been confirmed in hedgehogs, with the reported occurrence ranging from 9.8 % (4/41) (Skuballa et al., 2012) to 90 % (54/60) (Majerová et al., 2020). Furthermore, our results on *I. hexagonus* corroborate those from other studies investigating the occurrence of the bacteria in *I. hexagonus* collected from domestic species across GB with a

Table 2
Summary of the pan-*Borrelia* *q*PCR results for European hedgehog (*Erinaceus europaeus*) tissues and tick life stages tested. All positive samples were characterised as *B. afzelii*, excluding two samples where sequencing attempts failed ([c]).

	Tissue					Tick life stage		
	Ear	Muscle	Heart	Kidney	Urinary bladder	Larvae	Nymph	Adult (F)
Case 1[a]	+	–	–	–	–	–	+	–
Case 2[a]	–	+	+	+	+[c]	+	–	n.d.
Case 3[a]	+[c]	–	–	–	–	–	–	–
Case 4[b]	–	–	+	+	–	+	+	–

[a] animal infested with *I. hexagonus*.
[b] animal infested with *I. ricinus*.
[c] Sample failed to sequence
n. d., not done.

tested *Borrelia*-qPCR positivity of 0.4 % (1/224) and 0.8 % (3/386) in ticks retrieved from cats (n = 1855), and surveyed dogs, respectively (Abdullah et al., 2018; Davies et al., 2017). Notably in our dataset, the predominance of *I. hexagonus* compared with the small number of *I. ricinus* is of interest. While a high proportion of *I. hexagonus* was expected, given the known host-parasite association, a longitudinal study showed almost consistent co-feeding habits of the two tick species on hedgehogs in mainland Europe (Pfäffle et al., 2011). Jahfari et al. (2017) investigated 1131 *I. hexagonus* and 72 *I. ricinus* from 54 hedgehogs in Belgium, detecting *Borrelia* DNA in 23.0 % (260/1131) and 51.4 % (37/72) of ticks, respectively. Similarly, other studies from across Europe report a frequent occurrence of *I. ricinus* feeding on hedgehogs, and of the pathogen in both *I. ricinus* and *I. hexagonus*, as well as the hedgehog (Krawczyk et al., 2015; Skuballa et al., 2012).

Collectively, these findings indicate that *Borrelia* spp. infections in ticks and hedgehogs are more frequent in areas where *I. ricinus* is abundant and that the small number of *Borrelia*-DNA detections in this study is perhaps due to the minimal interaction between the two tick vectors. As a result, it is suggested that – differently from other countries in mainland Europe – in GB, there is a lower occurrence of the bacteria in hedgehogs and that *Borrelia* spp. may not be maintained in a transmission cycle between *I. hexagonus* and hedgehog populations, unless *I. ricinus* is locally abundant in the area. Indeed, Gray et al. (1994) primarily suggested the hedgehog as a *Borrelia* spp. reservoir host in an Irish habitat where *I. hexagonus* was absent. The infrequent detection of *I. ricinus* in hedgehogs may be due to high densities of alternative wildlife hosts for this tick species in GB (e.g. deer, game birds) in rural habitats (Gandy et al., 2021; Gilbert et al., 2012; Madden, 2021). Since *I. ricinus* is also found in higher densities in rural areas, this may contribute to the paucity of co-feeding with *I. hexagonus* in hedgehog populations utilising urban and suburban habitats (Hansford et al., 2023b), although this may differ if *I. ricinus* becomes more abundant in urban areas where hedgehogs occur. While post-mortem examinations were conducted on hedgehog carcasses as well preserved as possible, with likely interval from time of death to inspection of <72 h in the majority of cases, the frequency of ticks attached to carcasses may be lower than the counterpart live animals, which is a potential source of bias in our study findings.

Borrelia afzelii was the sole species characterised in our study, as per the identification in ticks collected from cats (Davies et al., 2017) and dogs (Abdullah et al., 2018) previously mentioned. Recently, a nationwide study investigating *Bbsl* infections in questing *I. ricinus* reported a lower detection of *B. afzelii* compared to *B. garinii* and *B. valaisiana* (Cull et al., 2021), supporting our inferences on the limited role that hedgehogs and *I. hexagonus* play in the wider transmission cycle of *Bbsl* in GB. Nevertheless, the 17 % (5/30) and 59 % (16/27) *Bbsl* species occurrence in *I. hexagonus* collected from eight hedgehogs and five badgers, respectively, from the same wildlife rehabilitation centre in South West England reported by Couper at al. (2010) could indicate that vector-host (s) relationships may change in limited areas, as badgers can also act as a reservoir for *B. afzelii* (Gern and Sell 2009). In comparison, these relationships may vary also in parts of Eastern Europe where a closely related hedgehog species, the Northern white-breasted hedgehog (*Erinaceus roumanicus*) is present (Dumitrache et al., 2013; Szekeres et al. 2019).

Our detection of *B. afzelii* in three cases shows that in GB: 1. the species occurs in hedgehogs and *I. hexagonus* larvae; 2. infected *I. ricinus* parasitise hedgehogs despite the small proportion of this tick species represented in the dataset; and 3. that infected larvae and nymphs of both tick species feed on this host. For Case 3, further investigation of the sample using the *B. miyamotoi* specific conventional PCR (Cull et al., 2021) could be considered, as only 0.2 % of the ticks analysed by Cull et al. (2021) tested positive for this bacterium, and the poor sequence quality obtained in our analysis hindered species characterisation. While it is noteworthy that the *B. afzelii* detections were from hedgehogs jn Autumn, given the limited sample size it is not possible to make meaningful inference regarding potential seasonality of infection without further investigation. Furthermore, these cases provide some evidence of potential host-vector interaction, although the direction of infection between them could not be determined as either host-to-vector or vector-to-vector transmission may occur (Toutoungi and Gern, 1993; Gray et al., 1994). When comparing experimental studies, transovarial transmission of *Borrelia* appears to be more frequent in *I. hexagonus* (15.8 %: 9/57 Gern et al., 1991) than in *I. ricinus*, in which transovarial transmission was detected at lower rates: 9.1 % (137/1500, Hauck et al., 2020), 1.2 % (39/3279, Duijvendijk et al., 2016), and considered a rare event (Richter et al., 2012). To further explore the potential for transovarial transmission in *I. hexagonus, Borrelia* DNA presence could be investigated in questing larvae by collecting them in the field either from hedgehog nests or by flagging in areas nearby.

Borrelia transmission from hedgehog host-to-larvae is also a plausible explanation for larval ticks of both species testing DNA-positive (Gern et al., 1997), since *I. ricinus* larvae can acquire spirochetes within two hours of feeding on rodents (Richter et al., 2012); however, nymph-to-larvae transmission due to co-feeding is an alternative explanation for Case 4 (see Table 2) (Voordouw, 2015). Interestingly, for the hedgehog with the greatest number of *I. hexagonus* ticks screened in our dataset (Case 3) (45 ticks, all three life stages) only localised (i.e., ear-positive only) *Borrelia* infection was detected. Further studies could focus on evaluating the time required by *I. hexagonus* to acquire *Bbsl* spirochetes from an infected host, which might provide useful insights into the blood meal necessary (e.g., full, partial, intermittent) for a tick to become infected.

The absence of significant inflammation consistent with borreliosis on microscopic examination of the DNA-positive hedgehog tissues suggests the animal can be infected without developing clinical disease. However, Lyme borreliosis is a challenging diagnosis with varied and non-specific clinical signs in domestic animals (e.g. neurological signs in horses (Imai et al., 2011); fever and lameness in dogs (Krupka and Straubinger, 2010)). Consequently, the paucity of reports of borreliosis in free-living wildlife does not rule out the potential for disease development in species known to be infected with the pathogen. Although it

remains unclear whether *Borrelia* has the potential to cause disease in individual hedgehogs, the low occurrence detected in this study indicates that the bacteria are unlikely to impact this species of conservation concern at a population scale.

The contribution of hedgehogs to the circulation of *Borrelia* and related public health risks appear to depend on the tick species the animal is infested with. Whilst in GB both tick species are known to bite people, *I. ricinus* is the more likely to be found on human hosts (Cull et al., 2020). Since *I. ricinus* is more frequently infected with *Borrelia* than *I. hexagonus* in GB (Cull et al., 2021), our results suggest that a bite by *I. hexagonus* poses a comparatively lower risk of infection than a bite by *I. ricinus*. Nevertheless, routine tick bite avoidance measures are recommended in the field and when handling wild animals, including wearing gloves and careful body screening to remove ticks (Due et al. 2013). Further defining the risk of exposure to infection in humans in GB could be achieved by conducting risk-based active surveillance, investigating host-vector relationships and the presence of the bacteria in vectors found in peri-domestic habitats.

5. Conclusions

To our knowledge, this is the first report of *Bb*sl infection in hedgehogs and *I. hexagonus* larvae in GB. However, the low occurrence of *B. afzelii* as the sole species characterised in both species suggests that the hedgehog and *I. hexagonus* play a limited role in the transmission cycle of *Borrelia* in GB. We hypothesise that the detected infection in host and vector may be a result of spill-over events from *I. ricinus*, which was not found to commonly infest this host in our dataset. These results are in contrast with similar studies conducted in mainland Europe, where both tick species are often found co-feeding on hedgehogs and the reported occurrence of the pathogen is higher in both vectors and the host. Our findings further indicate that the public health risk from *I. hexagonus* bites is lower compared to *I. ricinus* bites, and that for the hedgehog, the bacteria may not represent a health concern at either the individual or population level.

CRediT authorship contribution statement

Marco Vecchiato: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Becki Lawson:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Katharina Seilern-Moy:** Writing – review & editing, Resources, Investigation. **Mia L. White:** Investigation, Data curation. **Nicola Jones:** Investigation. **Faye Brown:** Investigation, Data curation. **Dylan Yaffy:** Writing – review & editing, Investigation. **Jolyon M. Medlock:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Kayleigh M. Hansford:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

none

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2025.102475.

Data availability

Data will be made available on request.

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