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Wild deer in the United Kingdom are a potential reservoir for the livestock parasite *Babesia divergens*

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ABSTRACT

Redwater fever is an economically important disease of cattle in the United Kingdom caused by the protozoan parasite *Babesia divergens*. Control efforts are dependent on accurate local historic knowledge of disease occurrence, together with an accurate appreciation of current underlying risk factors. Importantly, the involvement of red deer in the transmission of this pathogen in the UK remains unclear. We employed a polymerase chain reaction approach combined with DNA sequencing to investigate *Babesia* infections in livestock and red deer at a UK farm with a history of tick-borne disease. This revealed several *B. divergens*-infected cattle that were not displaying overt clinical signs. Additionally, 11% of red deer on the farmland and surrounding areas were infected with this parasite. We also found that 16% of the red deer were infected with *Babesia odocoilei*, the first time this parasite has been detected in the UK. The finding of *B. divergens* in the red deer population updates our knowledge of epidemiology in the UK and has implications for the effective control of redwater fever.

1. Introduction

Babesiosis is a globally important disease of humans and animals that has wide-ranging impacts on health and livelihood. Caused by various *Babesia* species, these intracellular parasites display high host specificity, with more than 100 different species identified so far. In the UK, redwater fever caused by *Babesia divergens* is of particular economic interest (Zintl et al., 2003). Although it primarily affects cattle, it can infect other species, including cottontail rabbits (*Lepus sylvaticus*) and reindeer (*Rangifer tarandus*) (Goethert & Telford, 2003; Langton et al., 2003). The parasite also has zoonotic potential (Gray et al., 2010), leading to at least one human fatality in the UK (Entrican et al., 1979a, b). However, symptoms of zoonotic infection are usually only evident in splenectomised or immunosuppressed individuals and it is the impact of this parasite on the cattle industry that is of primary concern (Kjemtrup & Conrad, 2000). Infected cattle may experience fever, anorexia, haemoglobinuria (which gives the disease its common name of "redwater"), anaemia and, in rare cases, neurological signs and death (Zintl et al., 2003). Globally, the pathogen is primarily transmitted by the tick vector *Ixodes ricinus*, although the parasite has also been associated with *Ixodes dentatus* in the USA (Goethert & Telford, 2003). Many *Babesia* species, including *B. divergens*, also demonstrate the capacity for transovarial transmission and persistence across life-cycle stages (Bonnet et al., 2007). This has implications for control efforts as the disease can be maintained between seasons even if removed from host mammals by treatment. In the British Isles, *I. ricinus* is widely distributed, with recent reports suggesting its range may be increasing (Cull et al., 2018). Efforts to control *B. divergens* in the UK have traditionally focused on identifying geographical areas of risk and management of grazing. Young cattle are then introduced into these risk areas in order to develop immunity, thereby promoting endemic stability in the locality (Pfeffer et al., 2018). This method relies on the well-recognised phenomenon of inverse age resistance to bovine babesiosis whereby calves up to a year of age display natural (innate) immunity to the disease (Zintl et al., 2005). This is thought to be largely

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driven by early nitric oxide (NO) production in the spleens of younger animals that contrasts with a delayed and less effective systemic response in older animals. As such, this response is unlikely to be due to maternal-associated passive immunity (Christensson, 1987). Infections tend to be characterised by low-level parasitaemia that can last for extended periods. Currently, babesiosis in UK cattle is primarily diagnosed on the basis of clinical signs alongside farm and animal history (Gray & Murphy, 1985; Zintl et al., 2014). Definitive confirmation of *Babesia* infection requires microscopic examination of stained blood smears and identification of pyriform bodies within erythrocytes. Several serological assays have also been developed to detect antibodies specific to *B. divergens* but their application has been limited by cost and practicality (Zintl et al., 2003). In the last decade, highly sensitive and specific molecular methods have emerged for parasite detection and species-level identification based on PCR amplification and sequencing of the parasite's 18S small subunit (SSU) ribosomal RNA gene (Lempereur et al., 2017). This gene is an attractive target for this approach due to its multi-copy nature in piroplasms, high level of within-species conservation, the presence of well-defined phylogenetically informative variable regions and the availability of many published sequences that assist in species classification. As well as amplicon sequencing-based methods proving more sensitive than *Babesia*-specific serological assays (Wang et al., 2015), they offer the advantage of directly detecting the pathogen rather than the antibody response that may only provide evidence of prior exposure.

It is unknown whether wild fauna play a role in spreading and maintaining babesiosis in UK cattle and so it is unclear whether this ecosystem compartment, which includes red deer, could confound efforts to control babesiosis. Some historical serological assays do indicate the potential for UK deer to be infected by *Babesia*, although the exact species involved are unknown (Latif & Adam, 1973; Adam et al., 1977). In continental Europe, roe deer (*Capreolus capreolus*) are the main vertebrate host for *Babesia venatorum* and are believed to be involved in the spread of this virulent zoonotic species (Michel et al., 2014; Zanet et al., 2014). Recently, sheep have also been identified as a reservoir for this species in the UK, having potential implications for the livestock industry (Gray et al., 2019). White-tailed deer (*Odocoileus virginianus*) are involved in the transmission of human infective *Babesia microti*, although they are not the primary reservoir (Piesman et al., 1979). This species is also maintained in an enzootic cycle involving voles and *Ixodes trianguliceps* in the UK, creating a potential reservoir for transmission to *I. ricinus* and new mammalian hosts (Bown et al., 2008). *Babesia divergens* has been confirmed by molecular assays in red deer (*Cervus elaphus*) in Ireland (Zintl et al., 2011) and red deer in continental Europe (Duh et al., 2005; Michel et al., 2014; Zintl et al., 2011) but it is unclear whether wild deer could be involved in the transmission of livestock babesiosis in the UK. Based on the importance of wild fauna in other settings, we hypothesise that native deer species could contribute to transmission of *B. divergens* in the UK. To investigate this premise, our study used a PCR-based approach targeting the 18S SSU rRNA locus to identify *Babesia* infections in cattle at a farm in the north-east of Scotland together with red deer in the surrounding area. This locality was selected because of a history of tick-borne disease in cattle, including cases of redwater and tickborne fever (http://theses.gla.ac.uk/id/eprint/8750). Culled red deer from the farm and surrounding areas were also examined for *Babesia* infection.

2. Materials and methods

At the farm site, which is located in Caithness, freshly clotted blood samples were obtained from 107 Luing cattle and frozen anticoagulated blood samples were obtained from 24 culled wild red deer in the immediate vicinity that share the same grazing land. At a further six unfenced hunting estates in the broader surrounding region, north of the Great Glen, 60 frozen clotted blood samples were obtained from culled wild red deer. At the hunting estates, blood samples were collected and stored in cryogenic vials and frozen at −20 °C on day of culling, prior to longer term storage at −80 °C. At the farm site, blood was collected using vacutainers and ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant. Blood samples were taken from the tail vein of cattle, which showed no sign of disease at the time of sampling. Similarly, the deer showed no overt evidence of disease and blood samples were obtained during post-mortem examination following routine culling. DNA was extracted from anticoagulated samples using a Wizard Genomic DNA Purification Kit (Promega, Madison, USA), according to the manufacturer's instructions. DNA was extracted from clotted blood with the same kit using a manufacturer-provided protocol for DNA extraction from mouse tissues that involved homogenisation before purification. PCR amplification of a diagnostic region of the 18S SSU rRNA gene was performed using a nested primer approach and previously described primers: Outer: BT1-F (5′-GGT TGA TCC TGC CAG TAG T-3′) and BTH-1R (5′-TTG CGA CCA TAC TCC CCC CA-3′) (Criado-Fornelio et al., 2003); and Inner: RLB-F2 (5′-GAC ACA GGG AGG TAG TGA CAA G-3′) and RLB-R2 (5′-CTA AGA ATT TCA CCT CTG ACA GT-3′) (Georges et al., 2001). GeneAmp PCR Buffer (Applied Biosystems, Waltham, USA) was utilised for PCR with a final concentration of 10 mM Tris-HCl, 50 mM KCl and 1.5 mM $MgCl_2$. The final reaction mix incorporated each of the four deoxynucleotides (New England Biolabs, Ipswich, USA) at 0.2 mM, each primer (Eurofins, Luxembourg) at 1 μM and AmpliTaq DNA Polymerase (Applied Biosystems) at 0.025 units per μl. Reactions were performed in a total volume of 20 μl. The conditions for the first round of PCR were an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of 94 °C for 45 s, 67 °C for 60 s and 72 °C for 60 s, with a final extension at 72 °C for 5 min. First round reactions were diluted 1:100 and then used as the template for the second round PCR using an initial step at 94 °C for 5 min, followed by 30 cycles of 94 °C for 45 s, 62 °C for 60 s, and 72 °C for 60 s, with another final extension at 72 °C for 5 min. Amplicons were separated using agarose gel electrophoresis and purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany), following the manufacturer's protocols. In order to minimise the risk of cross-contamination, steps were taken including the addition of a negative control with every PCR round, performing replicates of PCR rounds, strict adherence to decontamination protocols (including sodium hypochlorite and/or UV decontamination of pipetting equipment and surfaces) and spatial segregation of pre- and post-PCR activities. Amplicons were then sequenced by a commercial service (Eurofins) and trimmed using CLC Genomics Workbench (Qiagen). Amplicon sequences were characterised using BLAST to search the NCBI non-redundant database (https://blast.ncbi.nlm.nih.gov). All sequence data generated in this study were submitted to the GenBank database under the accession numbers MN563145-MN563173. To initially characterise the amplicons, sequences representing high-scoring BLAST 'hits' were identified from the NCBI database. For more accurate characterisation, all 18S SSU rDNA sequences unambiguously identified in the NCBI database as *B. divergens*, *Babesia odocoilei*, *B.* cf. *odocoilei* or *B. venatorum* were downloaded as FASTA files and aligned using Clustal Omega (Sievers & Higgins, 2014). These were then trimmed to the diagnostic region amplified by the 18S SSU rDNA PCR and identical sequences consolidated into a single representative sequence. Finally, the phylogenetic relationships between sequence variants were estimated using MrBayes with a GTR substitution model and default priors (Ronquist & Huelsenbeck, 2003). The bovine 18S sequence was used to root the tree.

3. Results

Our molecular analysis revealed that seven of the 107 cattle (6.5%) at the farm were PCR-positive for *Babesia*, whereas for the red deer, 11 of the 24 samples (46%) from the farm and 11 of the 60 samples (18.3%) from the broader area were positive. Subsequent analysis showed that all seven of the 18S SSU rDNA sequences from the cattle samples were identical, with a length of 371 nucleotides after primer trimming. These sequences were the same as 26 *B. divergens* sequences in the NCBI database, including AY046576. This entry has been suggested as an

appropriate reference sequence for the species (Gonzalez et al., 2014). Of the deer samples, nine of the sequences were 371 nucleotides in length and identical. BLAST analysis also identified them to be completely identical to the 26 *B. divergens* sequences in the NCBI database, including the suggested AY046576 reference. The remaining 13 sequences were quite distinct from *B. divergens*, being 370 nucleotides in length and differing from each other at only two nucleotide positions, namely 187 (T or G) and 217 (A or C). These showed high levels of similarity (> 95.16%) to various European *B. odocoilei* sequences. A phylogenetic tree was constructed using the data obtained from the deer and cattle samples and sequences identified as *B. divergens*, *B. odocoilei*, *B.* cf. *odocoilei* or *B. venatorum* from the NCBI database (Fig. 1). This confirmed that the amplicons generated were able to differentiate between these species and placed the putative *B. divergens* cattle and deer samples within the *B. divergens* clade. Potential *B. odocoilei* samples from deer were similarly placed into a clade containing *B. odocoilei* and *B. odocoilei*-like *B.* cf. *odocoilei* samples, although there was no bootstrap support for the distinction between *B. odocoilei* and *B.* cf. *odocoilei*.

4. Discussion

The PCR prevalence of *B. divergens* among the cattle on the farm was found to be low (6.5%) and beneath the seroprevalence rate of 11% recorded in a historic large-scale study in Scotland (Adam & Blewett, 1978). In that study, blood from more than 22,000 Scottish cattle were analysed to reveal that 27% of herds were infected (defined as seropositivity > 10%) and several foci of infection were present, including one in the north-east of Scotland in the Nairn area (Adam & Blewett, 1978). The set of sampled animals in the present study comprised the entire herd on the farm including calves, replacement stock and older breeding animals. In the UK, clinical babesiosis is primarily observed in spring and autumn (Jerram & Willshire, 2019). Although all stock had the opportunity to be exposed to ticks, as they had been previously out at pasture, the samples were collected in December, which is long after the normal spring/early summer peak in UK tick activity (Dobson et al., 2011). In addition, while the use of EDTA anticoagulated blood is considered standard for piroplasm PCR assays, a proportion of samples in the present study were clotted or coagulated which very likely reduced the sensitivity of the PCR reaction. As such, our estimate of the prevalence of *B. divergens* on the farm and in the surrounding areas is likely to be an underestimate. Despite this, it is important to note that all PCR-positive animals identified in the study were found to be healthy on clinical examination at the time of sampling, indicating that *B. divergens* can be detected in Scottish cattle herds with no apparent signs of disease. Our findings were somewhat surprising as a low prevalence of this parasite would tend to lead to a suspicion of enzootic instability in the locality. However, there had been no recent reports of redwater fever on the farm and the herd in question is 'closed', meaning all replacement stock are reared on the premises. It may be hypothesised that a serological investigation would have identified a far higher proportion of animals with exposure to *B. divergens*, and that the low PCR-prevalence is indeed most readily explained by the late season sampling period and the fact that clotted blood samples were used to generate template DNA.

Current UK control efforts rely on a risk management approach, primarily based on the identification of grazing areas with a prior history of clinical cases. However, veterinary surgeons across Scotland report that

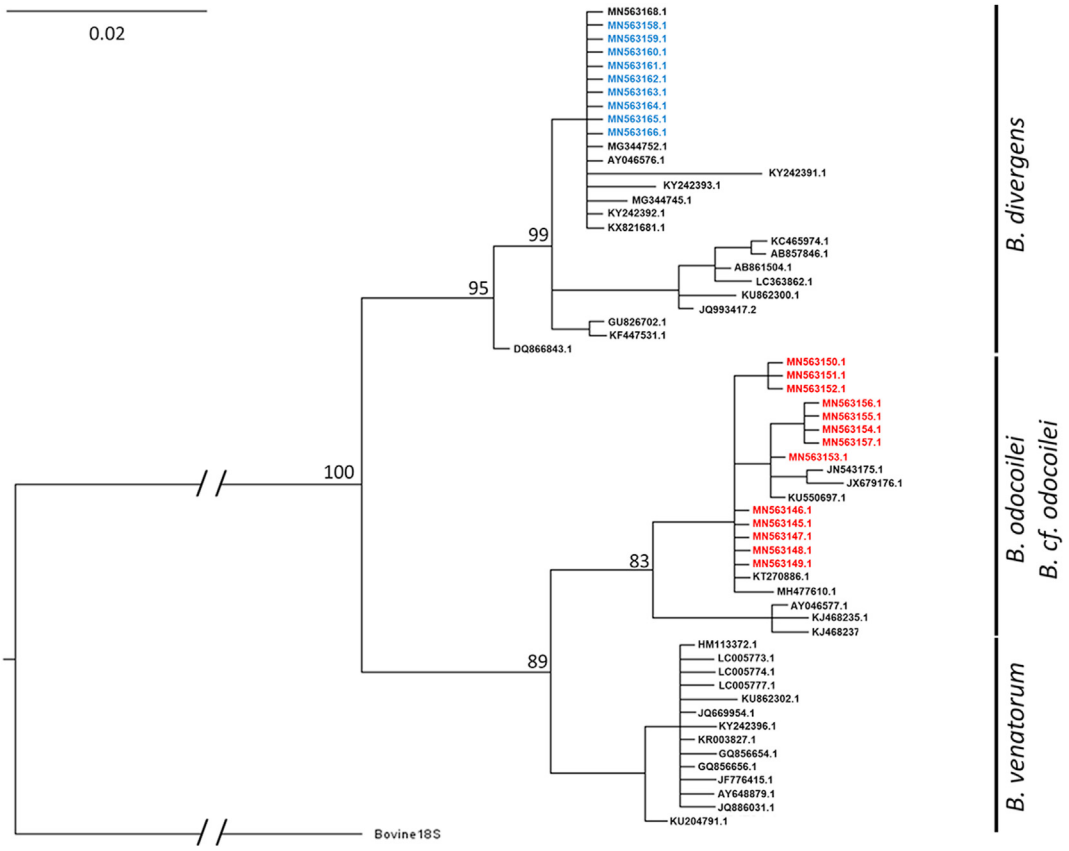

Fig. 1 A phylogenetic tree constructed using *Babesia* spp. 18S sequences amplified from Scottish cattle and red deer, in addition to sequences identified as *B. divergens*, *B. odocoilei*, *B.* cf. *odocoilei* or *B. venatorum* from the NCBI nucleotide database. The tree was estimated using MrBayes with a GTR substitution model and default priors. Identical sequences from the NCBI database were consolidated into single representative sequences. Bootstrap support is shown at the corresponding node if above 80. The *B. divergens* sequences detected in deer are shown in blue while the *B. divergens* sequences detected in cattle were identical to one another and a single representative sequence, MN563168.1, is shown. These were all identical to the *B. divergens* genome strain (AY046576.1). Sequences shown in red were identified as *B. odocoilei* or *B.* cf. *odocoilei* and were amplified from deer. The bovine 18S sequence was used to root the tree and tree lengths indicate genetic distance (scale shown)

sporadic cases of redwater fever do still occur and it may be hypothesised that additional but as yet unappreciated risk factors exist (http://theses.gla.ac.uk/id/eprint/8750). Importantly, our results confirm that it is possible that native red deer could serve as an overlooked reservoir for *B. divergens*, although the role they play in the transmission of redwater fever to livestock remains to be qualified and quantified. Wide-scale molecular screening of the red deer population may be required to accurately gauge the true prevalence and geographical range of *B. divergens* in the UK. It would also be worthwhile assessing whether the *B. divergens* population is genetically sub-structured based on these hosts and whether this is associated with differences in parasite virulence between host species. Red deer can occupy relatively expansive ranges in the UK, especially in Scotland, meaning there is the potential to spread pathogens between otherwise epidemiologically unconnected farms (Walker, 2015). Given the large geographical range of deer compared to both ticks and farmed cattle, it may be hypothesised that this could enable spread of *B. divergens* from an endemically stable area to fresh territory with a naïve cattle population. Moreover, it is also possible that the parasite could retreat to a tick-deer cycle if excluded from the cattle population by aggressive acaricidal therapy or depopulation, only to re-emerge at a later date and cause clinical disease in exposed naïve livestock. In addition to facilitating tick transport over long distances, studies have shown that deer play other important roles in the ecology of *I. ricinus*, such as providing sustenance for all active stages and providing a venue for tick mating (Jaenson et al., 2018). Consequently, deer are recognised as important maintenance hosts and, even if present at very low density, are known to be important drivers of tick abundance (Jaenson et al., 2018). Therefore, any future efforts to aggressively control *B. divergens* in the UK must consider the risk posed by infected native fauna.

The sylvatic cycle indicated by our findings also has implications for human health and as NHS inclusion criteria for human babesiosis include contact with cattle as a risk factor, our data suggest that contact with red deer in tick areas should also be considered. Future studies should therefore be aimed at quantifying the level of occupational risk in professions such as gamekeepers and stalkers, whose activities result in them being exposed to red deer and their habitat. Finally, our study also found several *B. odocoilei*-like sequences in the red deer population. In white-tail deer (*O. virginianus*) in North America, the host species in which *B. odocoilei* was originally identified, both sub-clinical infection and clinical disease has been documented (Perry et al., 1985). While this parasite has been shown to infect a wide range of cervine species, it is only known to cause severe clinical manifestations, including fatalities, in captive elk (*C. elaphus canadensis*) (Holman et al., 1994), woodland caribou (*R. tarandus caribou*) (Schoelkopf et al., 2005) and reindeer *(R. tarandus tarandus*) (Bartlett et al., 2009). The clinical significance of *B. odocoilei* in red deer is unknown and the importance of these parasites to the Scottish game industry remains unclear and requires further assessment. The presence of more than one species of *Babesia* in Scottish red deer highlights the fact that classical microscopy cannot be relied upon alone to identify *B. divergens* and that this may have precluded its identification previously. For this reason, future epidemiological studies should incorporate molecular methods for detecting and identifying this *Babesia* species.

5. Conclusions

The results of this study indicate that the epidemiology of *B. divergens* in the UK involves both the local cattle and red deer host compartments of the ecosystem. Thus, in combination with suitable tick habitat, the presence of red deer may need to be considered as a risk factor when identifying grazing localities that are hazardous for redwater fever. This has implications for the UK's current risk-based approach to babesiosis control that depends on a history of accurate diagnosis of infected livestock. Molecular screening of both the bovine and cervine population for presence of *B. divergens* may be required to fully elucidate risk of redwater fever.

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CRediT author statement

Alexander Gray: Formal analysis, Investigation, Writing - Original Draft. Paul Capewell: Methodology, Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing. Ruth Zadoks: Methodology, Resources, Writing - Review & Editing. Mark A. Taggart: Methodology, Resources, Writing - Review & Editing. Andrew S. French: Methodology, Investigation, Writing - Review & Editing. Frank Katzer: Conceptualization, Supervision, Funding acquisition, Writing - Review & Editing. Brian R. Shiels: Conceptualization, Supervision, Writing - Review & Editing. William Weir: Conceptualization, Supervision, Project administration, Funding acquisition, Writing - Review & Editing. All authors read and approved the final manuscript.

Data availability

The sequences generated in the study were deposited on GenBank under the accession numbers MN563145-MN563173.

Ethical approval

The Ethics and Welfare Committee of the University of Glasgow School of Veterinary Medicine approved blood sampling, consent and data management plans for the study (Ref. 15a/13).

Declaration of competing interests

The authors declare that they have no competing interests.

Editorial disclosure

Given his role as Co-Editor, Frank Katzer had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process of this article was delegated to Aneta Kostadinova.

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References

Adam, K.M.G., Beasley, S.J., Blewett, D.A., 1977. The occurrence of antibody to *Babesia* and to the virus of louping-ill in deer in Scotland. Res. Vet. Sci. 23, 133–138. https://doi.org/10.1016/S0034-5288(18)33144-8.

Adam, K.M.G., Blewett, D.A., 1978. A serological survey for *Babesia* in cattle in Scotland. II. The occurrence of antibody in the population and the distribution of infected herds. Ann. Trop. Med. Parasitol. 72, 417–428. https://doi.org/10.1080/00034983.1978.11719342.

Bartlett, S.L., Abou-Madi, N., Messick, J.B., Birkenheuer, A., Kollias, G.V., 2009. Diagnosis and treatment of *Babesia odocoilei* in captive reindeer (*Rangifer tarandus tarandus*) and recognition of three novel host species. J. Zoo Wildlife Med. 40, 152–159. https://doi.org/10.1638/2008-0011.1.

Bonnet, S., Jouglin, M., Malandrin, L., Becker, C., Agoulon, A., L'Hostis, M., Chauvin, A., 2007. Transstadial and transovarial persistence of *Babesia divergens* DNA in *Ixodes*

ricinus ticks fed on infected blood in a new skin-feeding technique. Parasitology 134, 197–207. https://doi.org/10.1017/S0031182006001545.

Bown, K.J., Lambin, X., Telford, G.R., Ogden, N.H., Telfer, S., Woldehiwet, Z., Birtles, R.J., 2008. Relative importance of *Ixodes ricinus* and *Ixodes trianguliceps* as vectors for *Anaplasma phagocytophilum* and *Babesia microti* in field vole (*Microtus agrestis*) populations. Appl. Environ. Microb. 74, 7118–7125. https://doi.org/10.1128/AEM.00625-08.

Christensson, D.A., 1987. Clinical and serological response after experimental inoculation with *Babesia divergens* of newborn calves with and without maternal antibodies. Acta Vet. Scand. 28, 381–392.

Criado-Fornelio, A., Martinez-Marcos, A., Buling-Saraña, A., Barba-Carretero, J.C., 2003. Molecular studies on *Babesia, Theileria* and *Hepatozoon* in southern Europe. Vet. Parasitol. 113, 189–201. https://doi.org/10.1016/S0304-4017(03)00078-5.

Cull, B., Pietzsch, M.E., Hansford, K.M., Gillingham, E.L., Medlock, J.M., 2018. Surveillance of British ticks: An overview of species records, host associations, and new records of *Ixodes ricinus* distribution. Ticks Tick-Borne Dis. 9, 605–614. https://doi.org/10.1016/j.ttbdis.2018.01.011.

Dobson, A.D.M., Taylor, J.L., Randolph, S.E., 2011. Tick (*Ixodes ricinus*) abundance and seasonality at recreational sites in the UK: Hazards in relation to fine-scale habitat types revealed by complementary sampling methods. Ticks Tick-Borne Dis. 2, 67–74. https://doi.org/10.1016/j.ttbdis.2011.03.002.

Duh, D., Petrovec, M., Avsic-Zupanc, T., 2005. Molecular characterization of human pathogen *Babesia* EU1 in *Ixodes ricinus* ticks from Slovenia. J. Parasitol. 91, 463–465. https://doi.org/10.1645/GE-394R.

Entrican, J.H., Alkhawam, H., Cook, I.A., Lancaster, W.M., Clark, J.C., Joyner, L.P., Lewis, D., 1979a. Babesiosis in man: Report of a case from Scotland with observations on the infecting strain. J. Infection 1, 227–234. https://doi.org/10.1016/S0163-4453(79)91219-2.

Entrican, J.H., Williams, H., Cook, I.A., Lancaster, W.M., Clark, J.C., Joyner, L.P., Lewis, D., 1979b. Babesiosis in man: A case from Scotland. Brit. Med. J. 2 https://doi.org/10.1136/bmj.2.6188.474, 474–474.

Georges, K., Loria, G.R., Riili, S., Greco, A., Caracappa, S., Jongejan, F., Sparagano, O., 2001. Detection of haemoparasites in cattle by reverse line blot hybridisation with a note on the distribution of ticks in Sicily. Vet. Parasitol. 99, 273–286. https://doi.org/10.1016/S0304-4017(01)00488-5.

Goethert, H.K., Telford, S.R., 2003. Enzootic transmission of *Babesia divergens* among cottontail rabbits on Nantucket Island, Massachusetts. Am. J. Trop. Med. Hyg. 69, 455–460. https://doi.org/10.4269/ajtmh.2003.69.455.

Gonzalez, L.M., Rojo, S., Gonzalez-Camacho, F., Luque, D., Lobo, C.A., Montero, E., 2014. Severe babesiosis in immunocompetent man, Spain, 2011. Emerg. Infect. Dis. 20, 724–726. https://doi.org/10.3201/eid2004.131409.

Gray, A., Capewell, P., Loney, C., Katzer, F., Shiels, B.R., Weir, W., 2019. Sheep as host species for zoonotic *Babesia venatorum*, United Kingdom. Emerg. Infect. Dis. 25, 2257–2260. https://doi.org/10.3201/eid2512.190459.

Gray, J.S., Murphy, T.M., 1985. An estimation of the prevalence and economic importance of clinical bovine babesiosis in the Republic of Ireland. Irish Vet. News, October 9–14.

Gray, J., Zintl, A., Hildebrandt, A., Hunfeld, K.-P., Weiss, L., 2010. Zoonotic babesiosis: Overview of the disease and novel aspects of pathogen identity. Ticks Tick-Borne Dis 1, 3–10. https://doi.org/10.1016/j.ttbdis.2009.11.003.

Holman, P.J., Craig, T.M., Crider, D.L.D., Petrini, K.R., Rhyan, J., Wagner, G.G., 1994. Culture isolation and partial characterization of a *Babesia* sp. from a North American elk (*Cervus elaphus*). J. Wildlife Dis. 30, 460–465. https://doi.org/10.7589/0090-3558-30.3.460.

Jaenson, T.G.T., Petersson, E.H., Jaenson, D.G.E., Kindberg, J., Pettersson, J.H.-O., Hjertqvist, M., et al., 2018. The importance of wildlife in the ecology and epidemiology of the TBE virus in Sweden: Incidence of human TBE correlates with abundance of deer and hares. Parasit. Vectors 11, 477. https://doi.org/10.1186/s13071-018-3057-4.

Jerram, L., Willshire, J., 2019. Babesiosis in the UK and approach to treatment. Livestock 24, 18–24. https://doi.org/10.12968/live.2019.24.1.18.

Kjemtrup, A.M., Conrad, P.A., 2000. Human babesiosis: An emerging tick-borne disease. Int. J. Parasitol. 30, 1323–1337. https://doi.org/10.1016/S0020-7519(00)00137-5.

Langton, C., Gray, J., Waters, P., Holman, P., 2003. Naturally acquired babesiosis in a reindeer (*Rangifer tarandus tarandus*) herd in Great Britain. Parasitol. Res. 89, 194–198. https://doi.org/10.1007/s00436-002-0737-x.

Latif, B.M., Adam, K.M., 1973. Antibody to *Babesia* in Scottish red deer (*Cervus elaphus*). Nature 241, 476–477. https://doi.org/10.1038/241476a0.

Lempereur, L., Beck, R., Fonseca, I., Marques, C., Duarte, A., Santos, M., et al., 2017. Guidelines for the detection of *Babesia* and *Theileria* parasites. Vector-Borne Zoonot. Dis. 17, 51–65. https://doi.org/10.1089/vbz.2016.1955.

Michel, A.O., Mathis, A., Ryser-Degiorgis, M.-P., 2014. *Babesia* spp. in European wild ruminant species: Parasite diversity and risk factors for infection. Vet. Res. 45, 65. https://doi.org/10.1186/1297-9716-45-65.

Perry, B.D., Nichols, D.K., Cullom, E.S., 1985. *Babesia odocoilei* Emerson and Wright, 1970 in white-tailed deer, *Odocoileus virginianus* (Zimmermann), in Virginia. J. Wildlife Dis. 21, 149–152. https://doi.org/10.7589/0090-3558-21.2.149.

Pfeffer, M., Król, N., Obiegala, A., 2018. 7. Prevention and control of tick-borne anaplasmosis, cowdriosis and babesiosis in the cattle industry. In: Garros, C., Bouyer, J., Takken, W., Smallegange, R.C. (Eds.), Ecology and control of vector-borne diseases. Wageningen Academic Publishers, The Netherlands, pp. 175–194. https://doi.org/10.3920/978-90-8686-863-6_7.

Piesman, J., Spielman, A., Etkind, P., Ruebush, T.K., Juranek, D.D., 1979. Role of deer in the epizootiology of *Babesia microti* in Massachusetts, USA. J. Med. Entomol. 15, 537–540. https://doi.org/10.1093/jmedent/15.5-6.537.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574. https://doi.org/10.1093/bioinformatics/btg180.

Schoelkopf, L., Hutchinson, C.E., Bendele, K.G., Goff, W.L., Willette, M., Rasmussen, J.M., Holman, P.J., 2005. New ruminant hosts and wider geographic range identified for *Babesia odocoilei* (Emerson & Wright, 1970). J. Wildlife Dis. 41, 683–690. https://doi.org/10.7589/0090-3558-41.4.683.

Sievers, F., Higgins, D.G., 2014. Clustal Omega. Curr. Protocols Bioinformatics 48. https://doi.org/10.1002/0471250953.bi0313s48.

Walker, M.D., 2015. In: Headhunting: The distribution of wild deer in Britain. British Naturalist Spring Edition.

Wang, G., Villafuerte, P., Zhuge, J., Visintainer, P., Wormser, G.P., 2015. Comparison of a quantitative PCR assay with peripheral blood smear examination for detection and quantitation of *Babesia microti* infection in humans. Diagn. Micr. Infect. Dis. 82, 109–113. https://doi.org/10.1016/j.diagmicrobio.2015.03.010.

Zanet, S., Trisciuoglio, A., Bottero, E., de Mera, I.G., Gortazar, C., Carpignano, M., Ferroglio, E., 2014. Piroplasmosis in wildlife: *Babesia* and *Theileria* affecting free-ranging ungulates and carnivores in the Italian Alps. Parasit. Vectors 7, 70. https://doi.org/10.1186/1756-3305-7-70.

Zintl, A., Finnerty, E.J., Murphy, T.M., de Waal, T., Gray, J.S., 2011. Babesias of red deer (*Cervus elaphus*) in Ireland. Vet. Res. 42, 7. https://doi.org/10.1186/1297-9716-42-7.

Zintl, A., Gray, J.S., Skerrett, H.E., Mulcahy, G., 2005. Possible mechanisms underlying age-related resistance to bovine babesiosis. Parasite Immunol. 27, 115–120. https://doi.org/10.1111/j.1365-3024.2005.00748.x.

Zintl, A., McGrath, G., O'Grady, L., Fanning, J., Downing, K., Roche, D., Casey, M., Gray, J.S., 2014. Changing incidence of bovine babesiosis in Ireland. Irish Vet. J. 67, 19. https://doi.org/10.1186/2046-0481-67-19.

Zintl, A., Mulcahy, G., Skerrett, H.E., Taylor, S.M., Gray, J.S., 2003. *Babesia divergens*, a bovine blood parasite of veterinary and zoonotic importance. Clin. Microbiol. Rev. 16, 622–636. https://doi.org/10.1128/CMR.16.4.622-636.2003.