Molecular Detection of Babesia divergens from an Outbreak of Babesiosis in Holstein Cows, England

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Abstract

Bovine babesiosis is a sporadic disease within the United Kingdom causing mortality and morbidity within the national herd. Despite numerous reports in cattle, there have been no published reports of its molecular characterization or genetic confirmation of the Babesia species since its first description in England. This study describes the molecular detection and species identification of Babesia divergens in a case of babesiosis from an outbreak of the disease in a farm in northern England. Phylogenetic analysis demonstrated that B. divergens 18S RNA sequence in England is 100% identical to B. divergens in Ireland and France. The sequence derived is publicly available and can be used to compare future cases of bovine babesiosis, especially if the pathogenesis of disease changes in response to the emergence of other Babesia species.

ABBREVIATIONS

B: Babesia; PCR: Polymerase Chain Reaction; UK: United Kingdom

INTRODUCTION

Bovine babesiosis is a tick-borne protozoan infection that causes morbidity and mortality in cattle. The disease is characterized by fever, listlessness, and dehydration resulting from haemolytic anaemia caused by the destruction of erythrocytes. This is evident in the red coloration in urine, hence the common name for the disease, red water. The most common Babesia species causing disease in Western Europe is Babesia divergens. Infections occur sporadically throughout Europe and may extend as far south as North Africa [1]. Its distribution is associated with that of its tick vector, Ixodes ricinus. However, additional species that can infect cattle include B. major, B. bovis, B. bigemina, B. ovata, B. occultans and B. venatorum (formly babesia sp.EU1) [2]. Co-infection with B. divergens and Anaplasmaphagocytophilum, the causative agent of tick-borne fever also transmitted by I. ricinus, appear to be very common, therefore blood samples from affected animals should be screened for both pathogens. In addition to infection in cattle, B. divergens is zoonotic with a number of documented cases reported across Europe in splenectomized individuals [1].

B. divergens was first described in England by McFadyean and Stockman [3], who named it Piroplasmida divergens. This initial study established that this species was morphologically distinct from the Babesia first described by Victor Babes and a babesia species detected in cattle in Devon [4] and now believed to be B. major [5]. Since this time B. divergens has been diagnosed in the United Kingdom (UK) by examination of Giemsa stained smears from cattle blood and size of the erythrocytic form of the parasite. A positive result is based on the presence of the trophozoite or merozoite forms of the parasite in erythrocytes and the length of the merozoite form has been used to distinguish different babesial species. Surprisingly, there have been no published reports of molecular characterisation of B. divergens in cattle from England or publically available DNA sequence from English derived cases. The only report from the UK is a fatal case of babesiosis in a reindeer (Rangifer tarandus tarandus) where a fragment of 18S...
ribosomal RNA gene was used to confirm the infecting organism [6]. A recent report has also used molecular methods to detect B. divergens in I. ricinus removed from a domestic dog [7]. There are B. divergens sequences from bovine babesiosis cases in Ireland where the incidence of clinical babesiosis is monitored and have been shown to be in decline [8]. In order to address this we have used molecular methods that have been applied to the detection of Babesias and Theilerias in England [9,10,11] to confirm infection with B. divergens in a recently described case of bovine babesiosis from a farm in England [12].

MATERIALS AND METHODS

DNA was extracted from 100 µl of EDTA treated blood taken from the first cow using the DN easy Blood and Tissue Kit (Qiagen, Manchester, UK) following the manufacturer’s instructions. Babesia parasites were detected using a pan-piroplasm PCR that amplifies a partial (423 base pair) fragment of the 18S ribosomal gene using primers PIRO-A (AATACCAATCCTGACACAGG) and PIRO-B (TCAAATGCAATGCCCCCAAC) [13]. Amplified products were sequenced using flanking primers and derived sequence edited in Laser gene version 12.1 (DNASTAR) and assigned to a particular species based on BLAST (NCBI) search, when agreement was ≥ 98% to sequences of known Babesia species in GenBank. Once identification was achieved, Neighbour-joining analysis was conducted using I MEGAv.6 [14]. Bootstrap values were calculated to test the robustness of the cluster using 1000 pseudoreplicates. Values greater than 70% were considered significant.

RESULTS AND DISCUSSION

In September of 2016, a pregnant female Holstein cow collapsed. A subsequent veterinary inspection suggested milk fever (hypocalcaemia) with a temperature of 37.2°C and the cow was treated with calcium intravenously. It subsequently gave birth to a still-born calf and shortly afterwards began to spasm and exhibited behavior including leg paddling and eye rolling. The legs became hyper-extended and the cow died shortly afterwards. A blood sample was taken from the animal. The legs became hyper-extended and the cow died shortly afterwards. A blood sample was taken from the animal. The legs became hyper-extended and the cow died shortly afterwards. A blood sample was taken from the animal. The legs became hyper-extended and the cow died shortly afterwards. A blood sample was taken from the animal. The legs became hyper-extended and the cow died shortly afterwards. A blood sample was taken from the animal.

Pan-piroplasm PCR successfully amplified a fragment of the correct size from DNA extracted from a blood sample obtained from the cow (Figure 1). The amplicon was sequenced to generate 364 base pairs with 100% identity to B. divergens from isolates derived from a number of European countries. Phylogenetic comparison with a range of B. divergens sequences from Scotland, Ireland and France confirmed the species designation and demonstrated that it was distinct from other small Babesia species (Figure 2). The sequence has been submitted to GenBank with accession number KY296360.

Numerous tests have been developed for diagnostic as well as epidemiological investigation of babesiosis in livestock. In Great Britain, the main approach is to match clinical evidence with detection of the parasite in stained blood smears [15]. Alternatively, serological detection is used such as Babesia-specific immunofluorescence antibody tests (IFAT). Babesia divergens was first described by McFadyean and Stockman [3] based on the morphology of the merozoite form, with dimensions of 1.5 - 2.0 by 0.4µm, leading to its designation as a small Babesia species. This has meant that past detection of babesia have been based purely on morphometric methods leading to uncertainty over the precise species designation [16,17]. However, more than 100 species of Babesia have been described [18], some...
of which are present in the UK tick population [7] and have a similar morphology during the erythrocytic stage of infection in their primary mammalian host. Also large Babesia species have been detected in the UK such as B. major [19] and B. motasi [10] that can infect livestock. A further issue has been the dearth of research activity on UK-endemic piroplasms since the 1980s.

CONCLUSION

B. divergens has been detected in English livestock for over 100 years although this is the first time that the infecting species has been confirmed by molecular methods. The tick vector, Ixodes ricinus is present across the country although disease is associated with areas of high tick abundance. There is evidence for endemic stability in livestock [20] and in the UK this is manifest as occasional outbreaks of bovine babesiosis when immunologically naïve adult cattle are moved onto fields where infected ticks are present. This is likely to be the case in the cattle herd reported in this study.

The application of piroplasm PCR-sequencing offers the opportunity to both detect and identify species of Babesia in cases of haemolytic anaemia in cattle and other livestock [21]. Further study is required to assess the sensitivity of this approach and its ability to detect both acute piroplasm infection and carrier status of British livestock.

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REFERENCES