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Short communication

Detection of *Borrelia burgdorferi* sensu lato DNA in the blood of wild bison from Białowieża Primeval Forest in eastern Poland

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Abstract

The aim of the present study was to investigate the occurrence of *Borrelia burgdorferi* sensu lato DNA in a group of 120 wild bison (*Bison bonasus*) from the Białowieża Primeval Forest in eastern Poland. The PCR technique revealed the presence of 16S RNA of *Borrelia burgdorferi* sensu lato in the blood of 16 out of 120 examined animals. DNA amplification by means of primers SC1 and SC2 gave a product with a size of 300-bp. The sequences of the PCR products obtained showed 100% homology with each other and 100% homology with *B. burgdorferi* s.l. 16S RNA gene DQ111061. Results of this study suggest that wild bison are important in maintaining agents of Lyme borreliosis, and that studies of reservoir competence of this species are indicated.

Key words: *Borrelia burgdorferi* sensu lato, wild bison, PCR, tick-borne diseases

Introduction

Lyme borreliosis is the most common tick-associated disease in the Northern hemisphere. The causative agent of the disease is *Borrelia burgdorferi*. In eastern Europe, *Borrelia burgdorferi* sensu lato infection is transmitted by ticks of the species *Ixodes ricinus* (Bröker 2012). Several animal species are reservoirs of this pathogen, making its epidemiology and trans-

mission cycle complex. *Borrelia* infection causes disease in humans and in certain animals such as ruminants, horses, and dogs (Barbour 1998). Various *Borrelia burgdorferi* genospecies are known to cause Lyme disease, five of which have so far been identified as pathogenic in Europe and three others, although they have been found in human cases, are of unknown pathogenicity at present (Jaenson et al. 2009). Small mammals and birds are the most important reservoir

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for *B. burgdorferi*. However, other vertebrates such as game animals, although incompetent reservoirs for the spirochete, act as a feeding source for ticks, potentially contributing to the dissemination of this infection (Barbour 1998). Previous studies in Poland have found a high proportion (16.4%) of wild bison with antibodies against *B. burgdorferi* (Siński et al. 1996). The aim of the present study was to demonstrate the presence of *Borrelia burgdorferi* sensu lato DNA in bison blood.

Materials and Methods

Animals and sampling

The study was conducted from 2009 to 2014. The research material was the blood taken from the heart of 120 wild bison from Białowieża Primeval Forest, immediately after they were killed (depopulation to maintain an appropriate number of animals in the Primeval Forest).

One EDTA-anticoagulated whole blood sample was collected from each animal for molecular examination.

DNA extraction and amplification

DNA was extracted from whole blood using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) or the Blood Mini DNA isolation kit (A&A Biotechnology, Gdańsk, Poland) according to the manufacturer's instructions. The amplification of *Borrelia* spp. DNA through PCR was performed using the forward primer SC1 (5'-GCT GTC AGT GCG TCT TAA-3'); and the reverse primer SC2 (5'-CTT AGC TGC TGC CTC CGT A-3'), which amplify a 300-bp region of the 16S rRNA gene of *B. burgdorferi* s.l. We used standard PCR conditions previously described by Skotarczak et al. (2005).

DNA sequencing

PCR products were purified using QIAquick spin columns (Qiagen, Germany) and eluted in 50 µl of Tris 10 mM, pH 7.6. DNA sequence was determined on both strands using the same primers employed for PCR at a DNA sequencing core facility (Research Institute, Polish Academy of Sciences, Warsaw, Poland). The DNA sequences were assembled and edited using SeqMan (DNASTAR, Lasergene, Madison, USA), and ClustalV alignments to the *B. burgdorferi* s.l. 16S RNA gene DQ111061 published in GenBank.

Results and Discussion

Of the 120 samples examined for *B. burgdorferi* s.l., 16 (13.33%) showed amplification products compatible with the positive control in PCR assays having a size of about 300 bp.

Legible sequences of DNA were obtained for all 16 PCR products. All showed 100% homology with the studied sequence of the 16S RNA gene fragment DQ111061.

The fact that *B. burgdorferi* s.l. DNA was detected in the blood from 13.33% of wild bison suggests that these animals are an important host of this spirochete.

In eastern Poland, Winiarczyk et al. (2007), Štefančíková et al. (2008), and Adaszek et al. (2010) have demonstrated antibodies against *B. burgdorferi* in serum samples taken from horses, pigs, cattle, and dogs. Detection of the genetic material of *B. burgdorferi* s.l. in bison blood confirms that wild bison not only act as sentinels for the presence of the pathogen in a given area (Skarphédinsson et al. 2005), but they could potentially become a reservoir for ticks which could eventually transmit the disease to people, pets and livestock. The clinical presence of this disease in this region of Poland has been diagnosed in dogs (Adaszek et al. 2010). Moreover, *B. burgdorferi* s.l. has been found in ticks by polymerase chain reaction (PCR) (Zygner et al. 2008, Dzięgiel et al. 2014).

In Poland, in 2011, a total of 9,157 cases of Lyme disease were registered in humans, 2% higher than in the previous year. The highest recorded rate for the eastern provinces of Poland is 75.5 per 100,000 humans, with 2,091 people being hospitalized due to this disease (Paradowska-Stankiewicz and Chrześcińska 2013).

The results of our study suggest that wild bison may be important in maintaining the agents of Lyme borreliosis, and that studies of reservoir competence of these species are indicated.

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