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Identification and molecular survey of *Borrelia burgdorferi* sensu lato in sika deer (*Cervus nippon*) from Jilin Province, north-eastern China

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ABSTRACT

Lyme disease caused by *Borrelia burgdorferi* sensu lato (s.l.) is a common disease of domestic animals and wildlife worldwide. Sika deer is first-grade state-protected wildlife animals in China and have economic consequences for humans. It is reported that sika deer may serve as an important reservoir host for several species of *B. burgdorferi* s.l. and may transmit these species to humans and animals. However, little is known about the presence of *Borrelia* pathogens in sika deer in China. In this study, the existence and prevalence of *Borrelia* sp. in sika deer from four regions of Jilin Province in China was assessed. Seventy-one blood samples of sika deer were collected and tested by nested-PCRs based on 16S ribosomal RNA (16S rRNA), outer surface protein A (*OspA*), flagenllin (*fla*), and 5S-23S rRNA intergenic spacer (5S-23S rRNA) genes of *B. burgdorferi* s.l. Six (8.45%) samples were positive for *Borrelia* sp. based on sequences of 4 genes. The positive samples were detected 18 for 16S rRNA, 10 for *OspA*, 16 for *fla* and 6 for 5S-23S, with the positive rates 25.35% (95% CI = 3.8–35.6), 14.08% (95% CI = 3.0–21.6), 22.54% (95% CI = 4.3–36.9) and 8.45% (95% CI = 1.7–22.9), respectively. Sequence analysis of the positive PCR products revealed that the partial 4 genes sequences in this study were all most similar to the sequences of *B. garinii* and *B. burgdorferi* sensu stricto (s.s.), no other *Borrelia* genospecies were found. This is the first report of *Borrelia* pathogens in sika deer in China. The findings in this study indicated that sika deer as potential natural host and may spread Lyme disease pathogen to animals, ticks, and even humans.

1. Introduction

Lyme disease (LD) is the most prevalent vector-borne disease in Europe, North America, and Asia, with an increasing annual incidence and geographic range (Bacon et al., 2008). More than 70 countries have reported cases of LD (Xie et al., 2015). LD is caused by bacteria spirochete, *B. burgdorferi* s.l., transmitted to humans most commonly through *Ixodes* tick vectors, causing a multi-system disease (Wang et al., 1999; Situm et al., 2000). To date, at least 20 genotypes of *B. burgdorferi* s.l. have been reported around the world (Baranton and De Martino, 2009; Ivanova et al., 2014). *B. burgdorferi* s.s. is mainly found in Europe and North America. *B. afzelii* and *B. garinii* are mainly found in Europe and Asia. *B. burgdorferi* s.l. is primarily distributed in northern China. *B. garinii* and *B. afzelii* are distributed in north-eastern and north-western China, and *B. burgdorferi* s.s. in southern China with low prevalence (Zhang et al., 1997).

Since first detection of *B. burgdorferi* from Hailin County, Heilongjiang Province, China in 1986, clinical cases and epidemiologic study demonstrated its sustaining till now (Ai et al., 1988). Subsequently, *Borrelia* spirochete has been detected and isolated from humans, cattle, horses, sheep, dogs, mice, cats, and camels from 29 Provinces in China using aetiology and serology methods (Wang et al., 2015).

The blacklegged tick (*Ixodes scapularis*) is one of the most important tick-borne disease vectors, responsible for transmitting

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a variety of zoonosis to humans, including Lyme disease, human granulocytic anaplasmosis (HGA), and human babesiosis (Zolnik et al., 2015). Not only do adult blacklegged ticks feed and mate on deer, but the population of deer and tick maintains a positive linear correlation (Moyer, 2015; Levi et al., 2012). When deer are eliminated from some habitats by hunting or fencing, the abundance of ticks typically decreases. Many studies have reported that *B. burgdorferi* s.l. could infect cervids, including white-tail deer (*Odocoileus yirginianus*), mule deer (*Odocoileus hemionus*), and sika deer mainly in the United States, Canada, Japan, and France (Chomel et al., 1994; Levi et al., 2012; Nieto et al., 2012). The sika deer (*Cervus nippon*) is a first-grade state-protected animal. There are less than one thousand wild sika deer in China, and it is considered to be a highly endangered species. Little information is available about *B. burgdorferi* s.l. infection in sika deer in China.

Although it is well known that *Ixodes* ticks feed on deer species, the role played by sika deer in the epidemiology of Lyme disease spirochetes and as a potential source for the spread of *B. burgdorferi* in China is not completely understood (Lane et al., 1991; Wilson et al., 1988). In the present study, blood samples of sika deer from four farms in Jilin Province of China were analysed for *B. burgdorferi* s.l. infections using nested PCR assays. The potential risk of *B. burgdorferi* infections in sika deer was assessed.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agriculture Sciences (NO. LVRIAEC2013-010). The sika deer were handled in accordance with good animal practices as set by the Animal Ethics Procedures and Guidelines of China.

2.2. B. burgdorferi *s.l. strains*

DNA from four standard *B. garinii* was provided by Dr. Fingerle (Nationales Referenzzentrum für Borrelien Max von Pettenkofer-Institut, LMU München) in Germany. *B. afzelii* strain Bo23 and *B. burgdorferi* s.s. strain B31 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA), Manassas, USA. Strain *B. garinii* SZ was provided by Lanzhou Veterinary Research Institute. (Baranton et al., 1992; Takada et al., 2001; Niu et al., 2010).

2.3. Sample collection

During June and July 2015, when the peak activities of adult ticks occur in north-eastern China. A total of 71 blood samples were collected from the jugular veins of *domestic* sika deer in Jilin Province (13 from Dongfeng County of Liaoyuan City, 15 from Tonghua City, 9 from Shuangyang region of Changchun City and 34 from Zuojia town of Jilin City). The sika deer were bred in the semi-free range system, of all samples were stored in tubes containing EDTA at 4 °C until DNA extraction.

2.4. Genomic DNA extraction

Genomic DNA was extracted from 300 μl blood using a commercial kit (DNeasy Blood & Tissue Kit, Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The DNA concentration was determined with a NanoDrop 2000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA), and stored at −20 °C until use.

2.5. Detection of Borrelia *sp.*

A nested PCR-specific detection of *B. burgdorferi* s.l. DNA was performed using eight independent sets of primers as described in Table 1, targeting the16S rRNA gene, *OspA* gene, *fla* gene, and 5S-23S rRNA gene (Marconi and Garon, 1992; Zhang et al., 2014; Postic et al., 1994; Guy and Stanek, 1991; Wodecka, 2007). The specificity of these primers was tested using standard *Borrelia* sp. genomic DNA samples, as well as other pathogens (*Babesia*, *Theileria* and *Anaplasma*), and these primers were then used to detect field blood samples of sika deer. PCR reactions were performed in a DNA thermocycler (BioRad, Hercules, CA, USA), and the PCR conditions were the same as those reported previously (Marconi and Garon, 1992; Zhang et al., 2014; Postic et al., 1994; Guy and Stanek, 1991; Wodecka, 2007). PCR products were separated by 1% agarose gel electrophoresis, and the positive amplicons were directly sequenced to single sequencing (GENEWIZ, Inc. Beijing China). The sequences obtained were compared aligned with previously published sequences deposited in GenBank by using BLAST (http:blast.ncbi.nim.nih.gov/blast.cgi), and the alignments of multiple sequences were executed in Florence Corpet (http://multalin.toulouse.inra.fr/multalin/).

2.6. Statistical analysis

The 95% confidence intervals (95% CIs) for the overall prevalence values of *B. burgdorferi* s.l. were calculated using IBM SPSS Statistics version 19.0.

3. Results

DNA extracted from 71 blood samples from sika deer was subjected to the nested PCRs, which is targeted the 16S rRNA, *OspA*, *fla*, and 5S-23S genes. According to the PCR results, in general, 6 samples were determined to be positive for *B. burgdorferi* s.l. infection, with the average positive rate of 8.45% (6/71, 95% CI = 1.7–22.9). The positive samples were detected 18 for 16S rRNA, 10 for *OspA*, 16 for *fla* and 6 for 5S-23S, with the positive rates 25.35% (95% CI = 3.8–35.6), 14.08% (95% CI = 3.0–21.6), 22.54% (95% CI = 4.3–36.9) and 8.45% (95% CI = 1.7–22.9), respectively. At least one gene of *B. burgdorferi* s.l. was detected in 6 (8.45%) blood samples out of the 71 examined (Table 2).

Sequence analysis of the positive PCR products based on these 4 genes in this study revealed that the sequences were most similar to the sequences of *B. garinii* (based on the sequences of 16S rRNA, *fla* and 5S-23S genes) and *B. burdorferi* s.s. (based on the sequences of 16S rRNA and *OspA* genes), no other *Borrelia* genospecies were found.

4. Discussion

China has a large population of sika deer. Since 1995, Jilin Province has established two core development zones in Shuangyang region of Changchun City and Dongfeng County of Liaoyuan City developing a domestic industry for sika deer. The herd number of sika deer in Shuangyang region was 195,000 in 2011, which accounts for 30% of the total deer in China (Jiang et al., 2012). In contrast, wild sika deer in China are not only first-grade state-protected animals, but are also highly endangered. There are less than one thousand wild sika deer in China, and some subspecies have been deracinated, including *Cervus nippon* grassianus and *Cervus nippon* hortulorum (Tan, 2011).

Previous studies have reported that *B. burgdorferi* s.l. could infect cervids, including black-tailed deer infected with *B. burgdorferi* s.s. in California (Lane et al., 2005), sika and roe deer (Capreolus