Research paper

First detection and molecular identification of *Borrelia* species in Bactrian camel (*Camelus bactrianus*) from Northwest China

Bintao Zhai[a], Qingli Niu[a,*], Zhijie Liu[a], Jifei Yang[a], Yuping Pan[a], Youquan Li[a], Hongxi Zhao[c], Jianxun Luo[a], Hong Yin[a,b,*]

[a] *State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Xujiaping 1, Lanzhou, Gansu 730046, PR China*
[b] *Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou 225009, PR China*
[c] *Agricultural College of Ningxia University, Yinchuan 750021, PR China*

ABSTRACT

Comprehensive epidemiological surveys for Lyme disease have not been conducted for the Bactrian camel in China. In this study, a total of 138 blood specimens collected from Bactrian camels from Zhangye City in Gansu Province and Yili and Aksu in Xinjiang Province, China, were examined for the presence of *Borrelia* spp. Species-specificity nested PCR based on the 5S-23S rRNA, *OspA*, *flaB* and 16S rRNA genes revealed that the total positive rate of *Borrelia* spp. was 3.6% (5/138, 95% CI = 0.2–17.9). These results were confirmed by sequence analysis of the positive PCR products or positive colonies. This is the first report of *Borrelia* pathogens in camels in China. Two *Borrelia* species that cause Lyme disease and one that causes relapsing fever were identified in the camel blood samples by sequencing. The findings of this study indicate that the Bactrian camel may serve as a potential natural host of Lyme disease and/or relapsing fever in China.

1. Introduction

Borrelia species are distributed throughout the world and are maintained in nature within various arthropod vectors and mammalian, avian or reptilian hosts (Brisson et al., 2012; Vollmer et al., 2011). In humans, *Borrelia* spp. are the causative agents of a major disease: Lyme borreliosis (LB) (mainly caused by *B. garinii*, *B. afzelii*, and *B. burgdorferi* sensu stricto). LB is also an important disease of domestic animals and wildlife worldwide. LB-group spirochetes, commonly known as *B. burgdorferi* s.l., cause one of the most significant natural zoonosis diseases that is carried and transmitted by *Ixodes* spp. ticks (Wodecka et al., 2010). There are at least 20 genospecies of *B. burgdorferi* s.l., which are classified based on their genetic differences. More than six of these genospecies have been reported in China (*B. burgdorferi* s.s., *B. garinii*, *B. afzelii*, *B. valaisiana*, *B. sinica* and *B. yangtze*) (Yu et al., 2016). *B. garinii* is the main genospecies and is distributed mainly in northern China, while *B. burgdorferi* s.s. is widely distributed in south China (Chen et al., 2013; Wan et al., 1998). *B. burgdorferi* s.l. has been detected in more than 20 mammalian species and seven genera of birds (Li, 2009). Studies have shown that in addition to humans, at least six other taxa of mammals (sheep, cattle, horses, dogs, cats and mice) and

two types of birds (seabirds and migratory birds) can be infected by *Borrelia* spirochetes in China (Keesing et al., 2009). Previous research used antibodies to detect the *Borrelia* spp. antigen within camel sera in Egypt and reported a positive rate of 47.8% (Helmy, 2000).

The genus *Camelus* contains three species: *Camelus dromedaries* (one-hump dromedary), *Camelus bactrianus* (two-hump Bactrian camel), and *Camelus bactrianus ferus* (two-hump Bactrian camel). Dromedaries are mainly found in the Arabian Peninsula, the Middle East, and parts of Africa, whereas Bactrian camels are mainly located in Central and Northeast Asia, Northern China, and Mongolia (Liu et al., 2015). *Camelus bactrianus ferus* is a new species of camel, and it is mainly distributed in China and Mongolia (Guo, 2009). Currently, there are an estimated 250,000 camels, which are mainly distributed in Xinjiang and the Inner Mongolian Autonomous Region in China (Feng, 2016). The Bactrian camel is an important economic livestock animal in northwest China.

To the best of our knowledge, little molecular information is available on the presence of *B. burgdorferi* s.l. complex in camels in China. The investigation of known *Borrelia* and new *Borrelia* spp. associations is important for animal health and the livestock industry. The aim of this study is to understand the role of camels as carriers and
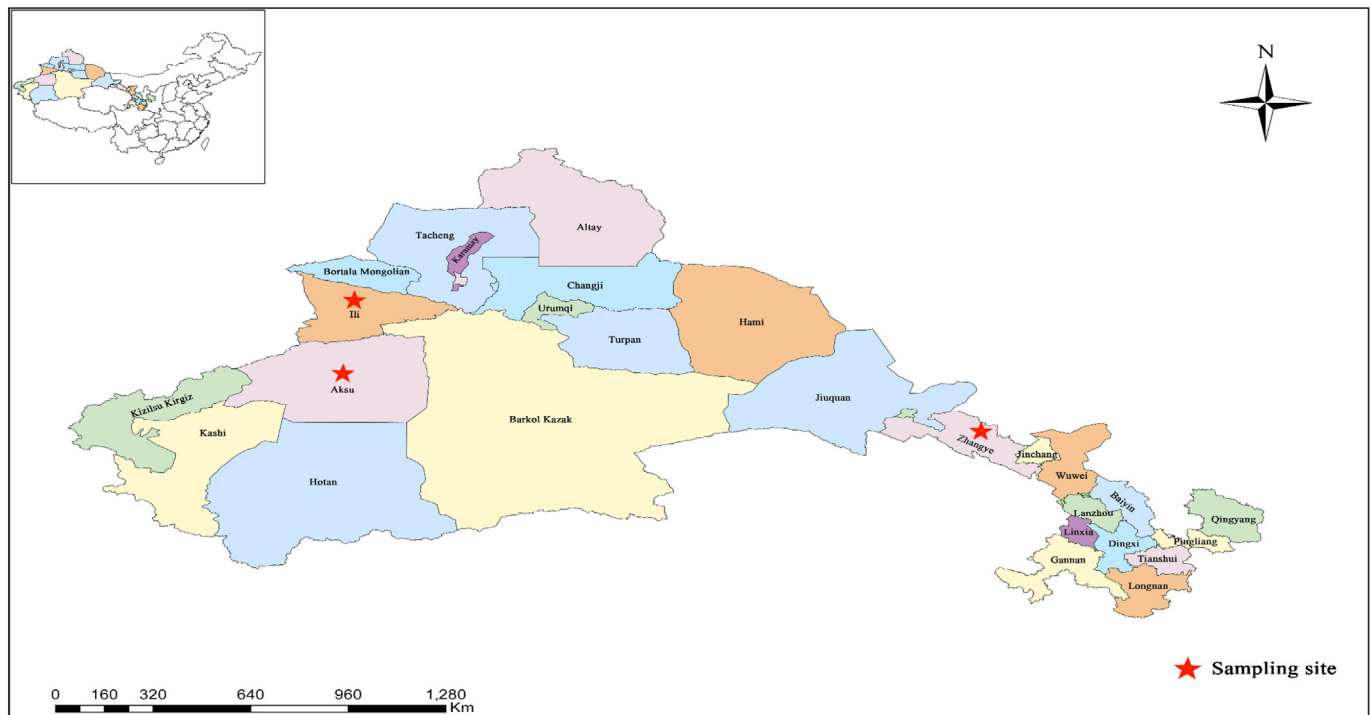
Fig. 1. Bactrian camel blood specimens were harvested in Northwest China (Gansu (Zhangye) and Xinjiang (Ili and Aksu)).

potential spreaders of Lyme disease in China. The exposure of camels to *B. burgdorferi* s.l. complex was investigated using PCR assay and sequencing. The sequences of the 5S-23S rRNA, *OspA*, *flaB* and 16S rRNA genes obtained from positive DNA samples were analysed, and the ticks that potentially act as vectors were discussed.

2. Materials and methods

2.1. Sample collection and DNA extraction

The Bactrian camel is free-choice grazing animal that inhabits desert regions. A total of 138 blood specimens from Bactrian camels were collected during May 2014 and November 2015 in two LB-endemic localities at three sites in northwest China (Gansu (Zhangye) and Xinjiang (Ili and Aksu)) (Fig. 1). Each blood sample was collected from the jugular vein of the camel into a sterile tube containing an anticoagulant (ethylene diamine tetraacetic acid, EDTA). Genomic DNA was extracted from individual specimens using a commercial QIAamp DNA Blood Kit (QIAGEN, Maryland, USA) and a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The extracted genomic DNA samples were then stored at −20 °C until use.

2.2. PCR amplification and sequence analysis

A nested PCR for the detection of *B. burgdorferi* s.l. was carried out using four independent sets of species-specific primers to amplify the 5S-23S rRNA, *OspA*, *flaB* and 16S rRNA genes, as described in previous studies (Zhang et al., 2014; Postic et al., 1994; Guy and Stanek, 1991; Wodecka, 2007; Ni et al., 2014; Zhai et al., 2017). The primer sequences are shown in Table 1. First-round PCR reactions were performed in a thermocycler (BioRad, Hercules, CA, USA) with a total volume of 25 μl containing 2.5 μl of 10× PCR buffer (Mg2+ Plus), 2.0 μl of each dNTP at 2.5 mM, 1.25 U of Taq DNA polymerase (TaKaRa, Dalian, China), 1.0 μl of template DNA, 1.0 μl of each primer (10 pmol), and 17.25 μl of distilled water. The PCR conditions were as follows: 4 min of denaturation at 94 °C; 35 cycles of 94 °C for 30 s, annealing for

30 s (annealing temperatures of primers are listed in Table 1), and 72 °C for 30–48 s (depending on amplicon size); with a final extension step at 72 °C for 10 min. Nested PCR reactions included 1 μl of the first-round PCR product as template for another 30 cycles with the same parameters and annealing temperature profile as described above and in Table 1. To avoid cross-contamination and sample carryover, pre- and post-PCR processing and PCR amplification was performed in separate rooms. *B. garinii* SZ genomic DNA (from Lanzhou Veterinary Research Institute) was used as a positive control, while distilled water was used as a negative control. PCR products were separated by 1.5% agarose gel electrophoresis, and some positive amplicons from the second round of PCR amplification were directly used for sequencing (GENEWIZ, Inc. Beijing China) or cloned into pGEM-T Easy vector (Promega, Madison, WI, USA). Positive colonies were then selected for PCR amplification and sequencing.

2.3. Bioinformatics analysis

All the sequences obtained in this study were subjected to BLAST search via the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using the BLASTn program. Multiple sequence alignment was executed in Florence Corpet (http://multalin.toulouse.inra.fr/multalin/). Phylogenetic analysis was performed using MEGA 6.06 software (Tamura et al., 2013). Phylogenetic trees of *Borrelia* spp. strains were constructed using all the sequences generated in this study and related sequences previously deposited in GenBank to show the relationships between different strains.

2.4. Statistical analysis

A 95% confidence interval (95% CI) for the overall prevalence value of each *Borrelia* spp. strain was calculated using IBM SPSS Statistics version 19.0.

2.5. Nucleotide accession numbers

All 8 sequences of *Borrelia* spp. 16S rRNA (including 3 sequences

Table 1
Sequences of the primers used in this study.

B. burgdorferi sensu lata target gene	Primer	Nucleotide sequence (5′-3′)	Annealing temperature (°C)	Amplicon size (bp)	Reference
16S	OuterF	GCGAACGGGTGAGTAACG	50	1360	Ni et al. (2014)
	OuterR	CCTCCCTTACGGGTTAGAA			
	InnerF	GAGGCGAAGGCGAACTTCTG	60.2	622	Zhai et al. (2017)
	InnerR	CTAGCGATTCCAACTTCATGAAG			
5S–23S	5S rRNA (rrf)	CGACCTTCTTCGCCTTAAAGC	57.6	412	Zhang et al. (2014)
	23S rRNA (rrl)	TAAGCTGACTAATACTAATTACCC			
	5S rRNA (rrf)IN	CTGCGAGTTCGCGGGAGA	57.3	253	Postic et al. (1994)
	23S rRNA (rrl)IN	TCCTAGGCATTCACCATA			
OspA	N1	GAGCTTAAAGGAACTTCTGATAA	52.5	561	Guy and Stanek (1991)
	CI	GTATTGTTGTACTGTAATTGT			
	N2	ATGGATCTGGAGTACTTGAA	53	352	
	C2	CTTAAAGTAACAGTTCCTTCT			
FlaB	OuterF	TGGTATGGGAGTTTCTGG	53.3	774	Wodecka (2007)
	OuterR	TCTGTCATTGTAGCATCTTT			
	InnerF	CAGACAACAGAGGGAAAT	54.7	604	
	InnerR	TCAAGTCTATTTTGGAAAGCACC			

Table 2
Prevalence of *Borrelia* spp. in camel blood samples from two Provinces in China assessed by the nested-PCRs.

Province	City		16S	5S–23S	*flaB*	*OspA*	At least one gene
Gansu	Zhangye	46	0 (0)	2 (4.3, 0.2–19.6)	0 (0)	2 (4.3, 0.2–19.6)	1(2.2, 0.1–13.8)
Xinjiang	Aksu	43	5 (11.6, 3.2–36.8)	2 (4.7, 0.2–19.8)	2 (4.7, 0.2–19.8)	0 (0)	4 (9.3, 2.1–28.9)
	Ili	49	3 (6.1, 1.3–20.1)	0 (0)	0 (0)	0 (0)	0 (0)
Total		138	8 (5.8, 1.1–17.2)	4 (2.9, 0.1–16.3)	2 (1.4, 0.1–10.6)	2 (1.4, 0.1–10.6)	5 (3.6, 0.2–17.9)
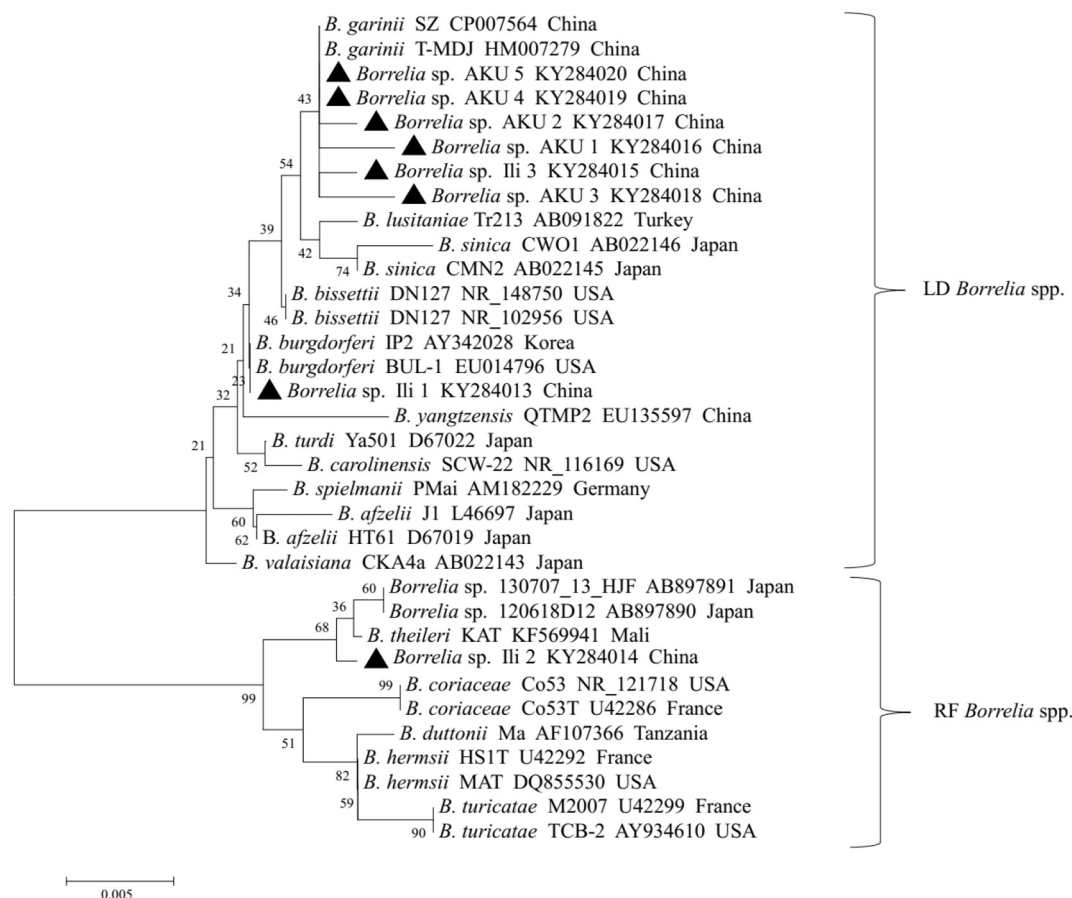
Fig. 2. Phylogenetic tree of the 16S rRNA gene sequences of Borrelia species obtained in the present study and those deposited in GenBank from different countries; accession numbers are shown after isolate names. The 16S rRNA gene sequences obtained in this study are indicated by bold triangles. The tree was inferred using the neighbour-joining method of MEGA 6.06; bootstrap values are shown at each branch point. Numbers above the branch reflect bootstrap support from 500 replications. All sites of the alignment containing insertions-deletions or missing data were eliminated from the analysis (option "complete deletion").
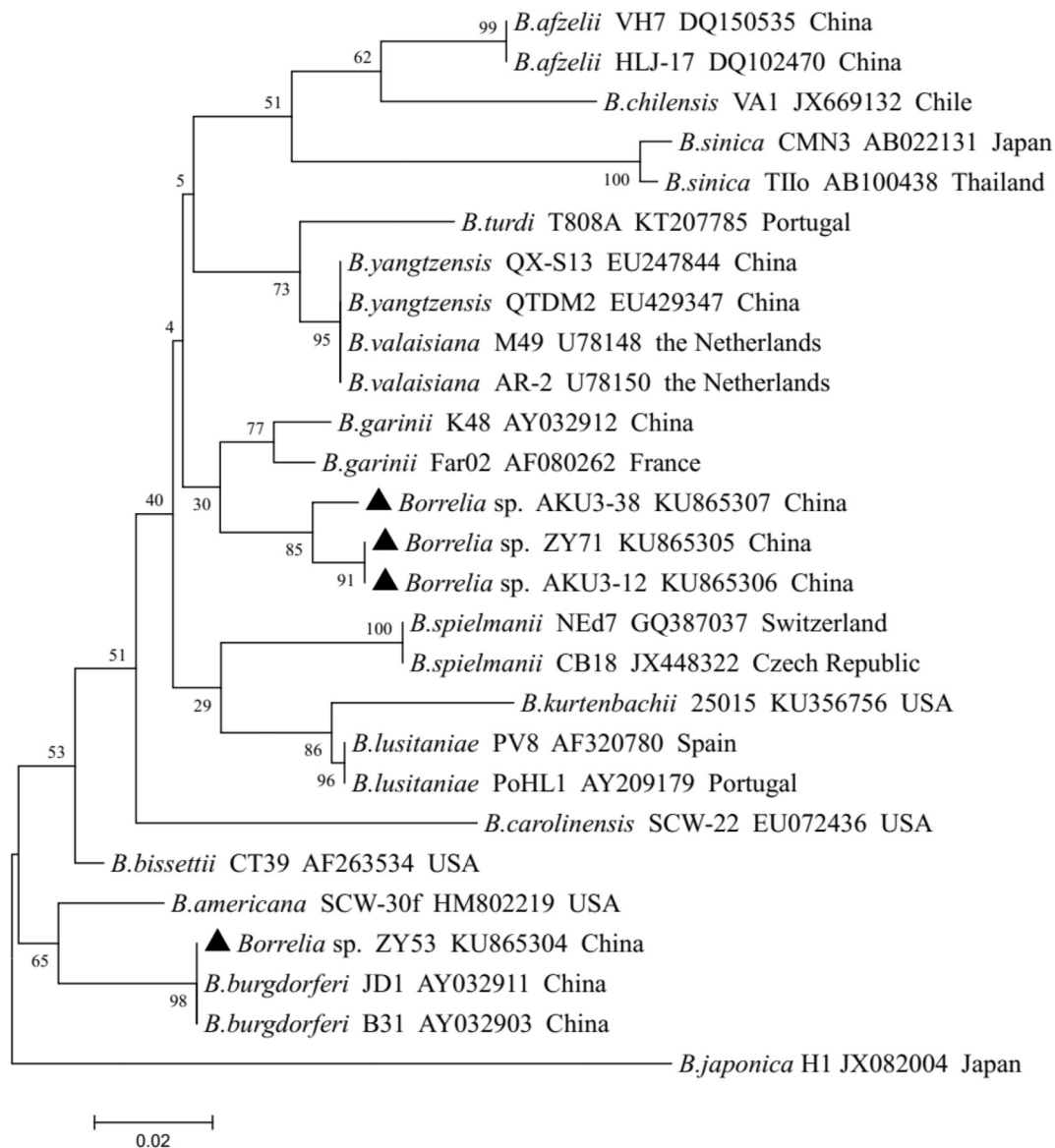
Fig. 3. Phylogenetic tree of the 5S-23S rRNA gene sequences of Borrelia species obtained in the present study and those deposited in GenBank from different countries; accession numbers are shown after isolate names. The 5S-23S rRNA gene sequences obtained in this study are indicated by bold triangles. The tree was inferred using the neighbour-joining method of MEGA 6.06; bootstrap values are shown at each branch point. Numbers above the branch reflect bootstrap support from 500 replications. All sites of the alignment containing insertions-deletions or missing data were eliminated from the analysis (option "complete deletion").

from Ili City and 5 sequences from Aksu City) were deposited in GenBank under the following accession numbers: KY284013-KY284020 (among them, AKU1 = AKU3-12, AKU3 = AKU3-14, AKU4 = AKU3-38, and AKU5 = AKU3-45). The sequences of the *Borrelia* spp. 5S-23S rRNA gene, including 2 sequences from Zhangye City and 2 sequences from Aksu City, were deposited in GenBank under the following accession numbers: KU865304-KU865307. Two sequences of the *Borrelia* spp. *flaB* gene from Aksu City were deposited in GenBank with the following accession numbers: KU865318-KU865319. Two sequences of the *Borrelia* spp. *OspA* gene from Zhangye City were deposited in GenBank with the following accession numbers: KU865317 and KY328701.

3. Results

3.1. PCR detection of Borrelia burgdorferi spirochetes from Bactrian camel blood samples

The blood samples collected from a total of 138 Bactrian camels in the field two Chinese provinces were screened for the presence of *Borrelia* spp. by nested PCRs based on four gene loci. The samples were amplified, and the PCR products had lengths of 253–622 bp for the four genes. The results of the nested PCR amplification for positive sample screenings are summarized in Table 2. Of these specimens, 4 tested positive for 5S-23S rRNA, 2 tested positive for *OspA*, 2 tested positive for *flaB* and 8 tested positive for 16S rRNA, with positive rates of 2.9% (95% CI = 0.1–16.3), 1.4% (95% CI = 0.1–14.9), 1.4% (95% CI = 0.1–10.6) and 5.8% (95% CI = 1.3–18.5), respectively. (The sample ZY53 was detected from both 5S-23S rRNA and *OspA*, the samples AKU3-12 and AKU3-18 were detected from both 5S-23S rRNA and 16S rRNA, and the samples AKU3-14 and AKU3-45 were detected
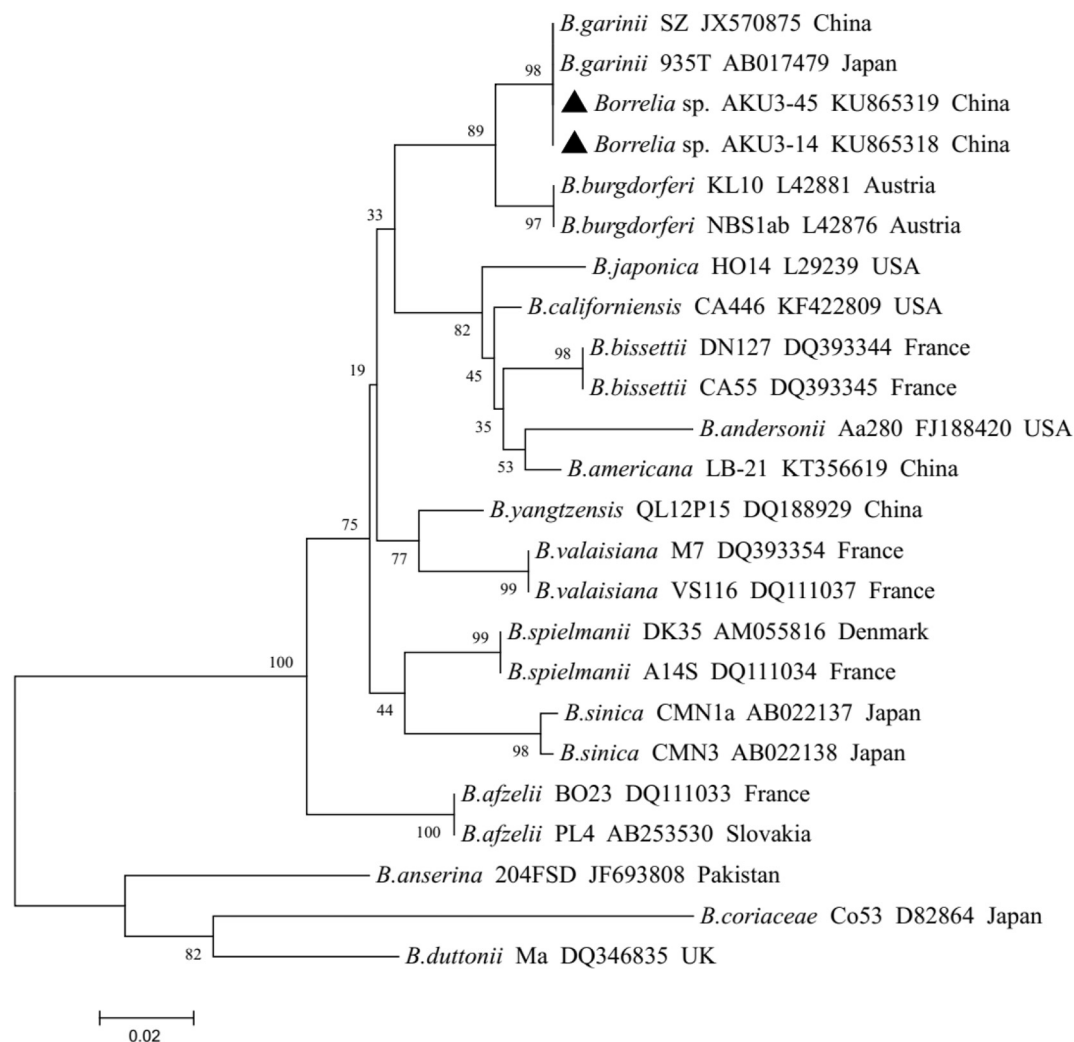
Fig. 4. Phylogenetic tree of the *flaB* gene sequences of Borrelia species obtained in the present study and those deposited in GenBank from different countries; accession numbers are shown after isolate names. The *flaB* gene sequences obtained in this study are indicated by bold triangles. The tree was inferred using the neighbour-joining method of MEGA 6.06; bootstrap values are shown at each branch point. Numbers above the branch reflect bootstrap support from 500 replications. All sites of the alignment containing insertions-deletions or missing data were eliminated from the analysis (option "complete deletion").

from both *flaB* and 16S rRNA). At least one *B. burgdorferi* s.l. gene was detected in 5 of the 138 blood samples examined (3.6%, 95% CI = 0.2–17.9).

3.2. Sequence and phylogenetic analysis

Sequence analysis of the positive PCR products of the 4 genes assayed in this study revealed that the sequences were most similar to those of *B. garinii* based on the 16S rRNA, 5S-23S rRNA and *flaB* gene sequences (100%, 85–100%, and 96–100% identity, respectively) (GenBank accession numbers: CP007564, DQ102468 and CP007564) and were also similar to *B. burgdorferi* (100%, 100%, and 98–100% identity, respectively) (GenBank accession numbers: AF210138, KP400556 and CP009657). Interestingly, a novel *Borrelia* genospecies (GenBank accession number: KY284014) was identified from the Ili region, which had a high identity with *B. theileri* KAT (99.5%, GenBank accession number: KF569941), which was detected from *Rhipicephalus geigyi*, and with *Borrelia* sp., with 99.8% identity (GenBank accession number: AB897890), which was detected from *Haemaphysalis* ticks collected from wild sika deer (*Cervus nippon yesoensis*) from Hokkaido, Japan, based on 16S rRNA.

Phylogenetic trees were constructed based on the identified *Borrelia* spp. 16S rRNA (*n* = 8), 5S-23S rRNA (*n* = 4), *flaB* (*n* = 2) and *OspA*

(*n* = 2) gene sequences by the neighbour-joining method using the software MEGA 6.06 (Fig. 2-5). The phylogenetic tree based on 16S rRNA sequences indicated that the 16S rRNA gene sequences (5 from Aksu, 3 from Ili) from our study formed three distinct clades. The five AKU (Aksu) strains cluster within a sub-clade of one of three main clades, forming a sister clade with the *B. garinii* 16S rRNA sequences from China. Interestingly, the strains from the Ili area belonged to two different clades, with two sequences of *B. garinii* and *B. burgdorferi* belonging to the LD *Borrelia* spp. group and one sub-clade of *B. theileri* belonging to RF *Borrelia* spp. group. In general, the results show the presence of high heterogeneity among the 16S rRNA sequences of the 8 different *Borrelia* species strains (Fig. 2). The 5S-23S rRNA sequences formed two distinct clades: 4 strains (2 from Aksu and 2 from Zhangye) formed two distinct sub-clusters in the *Borrelia* 5S-23S rRNA phylogenetic tree (Fig. 3). The 5S-23S rRNA sequences from the 2 Aksu strains (AKU3–12 = AKU2 of 16S rRNA, AKU3–38 = AKU4 of 16S rRNA) and one Zhangye *Borrelia* spp. strain belong to one clade of the same branch, which are sister to *B. garinii* 16S rRNA. One Zhangye strain of the 5S-23S rRNA sequence was located within the same branch (Fig. 3). A phylogenetic tree was constructed based on all the *Borrelia flaB* sequences deposited in GenBank and two sequences (AKU3-14 = AKU3 of 16S rRNA and AKU3-45 = AKU5 of 16S rRNA) obtained in this study. The *flaB* sequences from *Borrelia* spp. formed two main clades. The
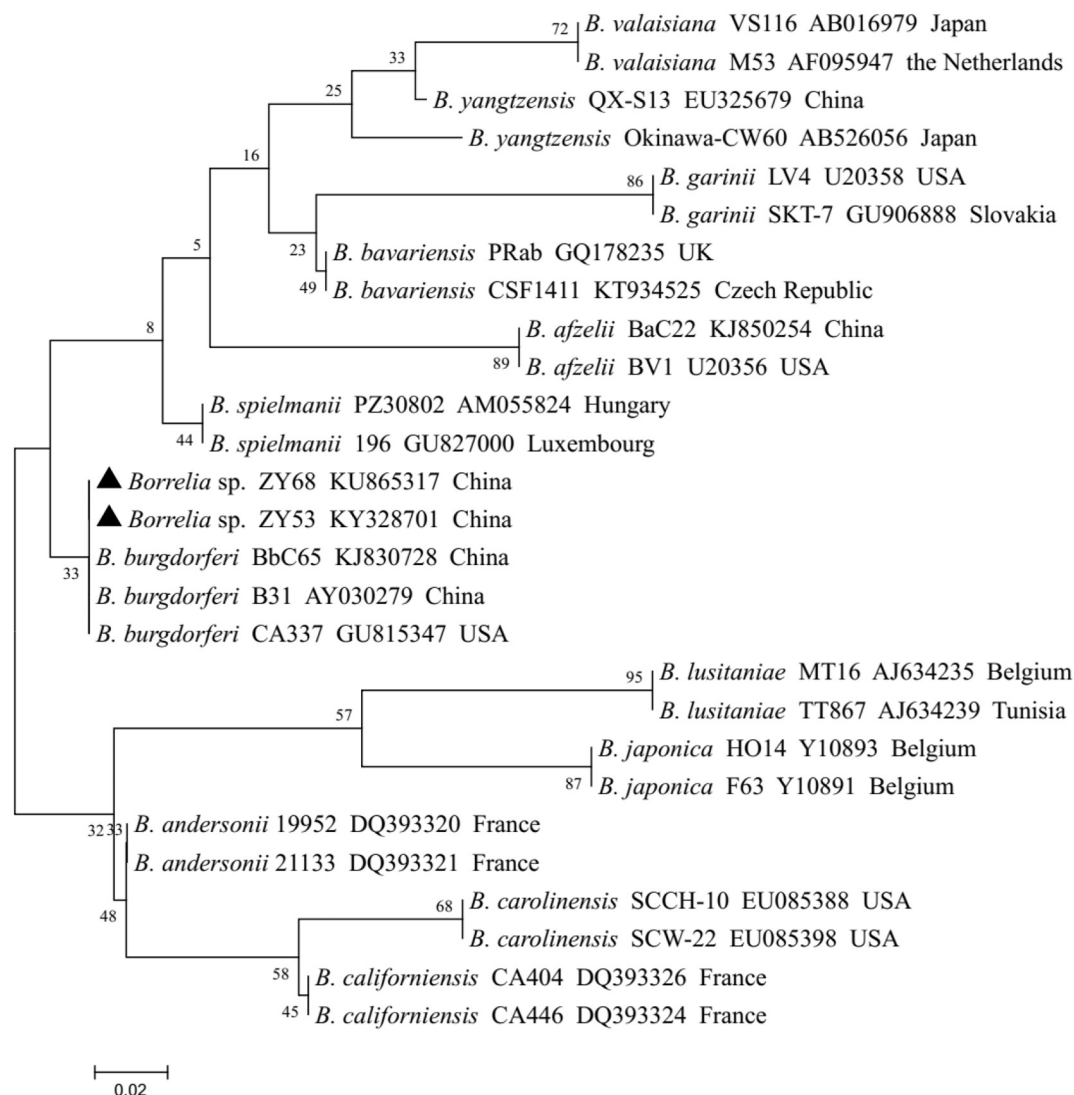
Fig. 5. Phylogenetic tree of the *OspA* gene sequences of Borrelia species obtained in the present study and those deposited in GenBank from different countries; accession numbers are shown after isolate names. The *OspA* gene sequences obtained in this study are indicated by bold triangles. The tree was inferred using the neighbour-joining method of MEGA 6.06; bootstrap values are shown at each branch point. Numbers above the branch reflect bootstrap support from 500 replications. All sites of the alignment containing insertions-deletions or missing data were eliminated from the analysis (option "complete deletion").

sequences of the two Aksu strains clustered together with the *B. garinii flaB* sequences (Fig. 4). Two distinct clades were formed from the sequences of the *Borrelia* spp. *OspA* phylogenetic tree. The sequences of two strains from Zhangye clustered with the *B. burgdorferi OspA* sequences (Fig. 5).

4. Discussion

In China, Lyme disease is caused by various *Borrelia* spirochetes. Many of these agents are highly pathogenic to both humans and animals (Liu et al., 2000). Previous studies reported the prevalence of *Borrelia* in field-collected blood samples from cattle, sheep, dogs, rabbits and rats from different areas in China. These studies primarily used serological detection methods and showed that the distribution of *Borrelia* varied considerably in the different areas (Hou et al., 2010; Wan et al., 1998).

The areas of Zhangye City, Gansu Province, and of Ili City and Aksu City of Xinjiang Province all include desert regions and are located along the Old Silk Road, halfway between Eastern Asia and Europe, in areas where international livestock trade and travel were frequent (Takada et al., 2001). Bactrian camels were important transportation

for trade and travel in the desert within these regions.

The Bactrian camel can harbour and spread many zoonoses, such as Middle East respiratory syndrome coronavirus (MERS-CoV), *Anaplasma, Toxoplasma gondii, Onchocerca, Trypanosoma evansi,* and *Parabronema skrjabini* (Liu et al., 2015; Luo, 2012; Wang et al., 2013; Yang et al., 2004; Yang et al., 2008). Ticks are one of the most significant vectors of *Borrelia burgdorferi* s.l. and RF (relapsing fever). Domestic animals, rodents, and many other wild animals host ticks, and animals bitten by infected ticks can acquire the pathogen and serve as natural reservoirs.

The detection of *B. burgdorferi* s.l. using PCR is an alternative method that can be used to improve the control and prevention of Lyme disease. According to the nested PCR results, 138 field-collected blood samples assayed with primers targeting the 16S rRNA, 5S-23S rRNA, *flaB*, and *OspA* genes revealed 8 (5.8%), 4 (2.9%), 2 (1.4%) and 2 (1.4%) positive samples, respectively, from three regions of two provinces in China where these camels live. According to our knowledge, this is the first report of *Borrelia* spp. infection in camels in China, indicating their reservoir role in the maintenance of this organism in the environment. The 16S rRNA gene sequences of *Borrelia* spp. detected from the Aksu region had the highest infection rate (11.6%), followed by the Ili region (6.1%). The genetic identity of *B. burgdorferi*

spirochetes can be clarified by their differential reactivity with genos-pecies-specific PCR primers targeting the 5S-23S rRNA intergenic spacer amplicon gene. Genetic heterogeneity should be further classi-fied by analysing longer sequence data among *B. burgdorferi* strains that have been previously identified as the same genospecies of atypical strains of *Borrelia* spirochetes (Mathiesen Jr et al., 1997; Postic et al., 1998). Moreover, the 16S rRNA gene was detected at a higher positive rate in blood examined for *Borrelia* spirochetes than other genes in previous research (Wodecka et al., 2010). Our study showed that the infection rate of these genes decreased in the order 16S rRNA > 5S-23S rRNA > *flaB* > *OspA*.

Two *Borrelia* species, *B. garinii* and *B. burgdorferi* s.s., were identi-fied, and *B. garinii* was found to be widely distributed in camels in China. In the present study, *B. garinii* and *B. burgdorferi* s.s. were identified in camels from Aksu and Ili in Xinjiang Province. Sequence and phylogenetic analysis revealed that those isolates were closely re-lated to the corresponding genotypes based on 16S rRNA gene with high sequence similarities (99.4%–100%, GenBank accession numbers: CP007564; 100%, GenBank accession numbers: AY342028), although the bootstrap values of the phylogenetic tree were relatively low. This finding suggested the genetic diversity of *B. garinii* and *B. burgdorferi* s.s in different hosts and geographic locations. Interestingly, the sequen-cing of cloned PCR products from the 16S rRNA gene of *Borrelia* spp. from the Ili region showed the presence of a new *Borrelia* species be-longing to the relapsing fever group. The 16S rRNA gene sequence of *Borrelia* sp. obtained from camel has a 99.5%, 99.8% and 99.8% simi-larity to the gene of *B. theileri* KAT strain, *Borrelia* sp. 120618D12 and *Borrelia* sp. 130707_13_HJF, respectively (GenBank accession numbers: KF569941, AB897890 and AB897891). *B. theileri* belongs to the RF *Borrelia* spp. group and is the causative agent of bovine borreliosis. It was initially identified in cattle and subsequently in goats, sheep and deer from Africa, South America, Mexico and Australia (L, 1903; Mathiesen Jr et al., 1997). Most of the RF *Borrelia* spp. are transmitted by soft-bodied ticks, but *B. theileri* is found in hard-bodied ticks and is transmitted by *Rhipicephalus* spp., including *R. annulatus*, *R. decoloratus*, *R. microplus* and *R. evertsi* (Barbour et al., 2005; Smith et al., 1985; Trees, 1978). This study provides the first report of *B. theileri* in camel blood samples in China.

At present, *B. burgdorferi* has been isolated from nine *Ixodes* ticks: *I. acutitarsus*, *I. persulcatus*, *I. granulatus*, *H. longicornis*, *H. bispinosa*, *H. concinna*, *H. formosensis*, *Boophilus microplus* and *D. silvarum* (Niu et al., 2009). A previous study reported that the *Borrelia* isolates were isolated from *D. marginatus* collected from camels in Xinjiang, China, and these isolates were genetically identified as *B. burgdorferi* sensu stricto (Wang et al., 2015). The blood samples from Bactrian camels in this study were donated by Dr. Li, who reported that there are ticks available to be collected from these Bactrian camels that have been identified as be-longing to *H. asiaticum*, *H. dromedarii*, *R. sanguineus* group, and *D. niveus* (Li et al., 2015). Thus, these tick species might act as potential vectors to carry and transfer *Borrelia* spp. that cause camel borreliosis in China. Further study is required to determine whether these ticks are compe-tent vectors for *Borrelia* spp.

In conclusion, we successfully identified infection with *Borrelia* spirochetes from camel blood samples from different geographic loca-tions of Gansu Province and Xinjiang Province in China. *B. garinii* and *B. burgdorferi* s.s. were highly prevalent in the sampling areas of the two provinces surveyed. Further studies concerning the prevalence of *Borrelia* spp. groups for both Lyme disease and relapsing fever should be performed to confirm the presence of these different *Borrelia* species in camels within China. Our findings suggest that *Borrelia* infection in camels could potentially present a concern for public health. More detailed and widespread monitoring of tick populations and the screening for *Borrelia* in a greater variety of hosts are warranted in future studies.

References

Barbour, A.G., Putteet-Driver, A.D., Bunikis, J., 2005. Horizontally acquired genes for purine salvage in *Borrelia* spp. causing relapsing fever. Infect. Immun. 73 (9), 6165–6168.

Brisson, D., Drecktrah, D., Eggers, C.H., Samuels, D.S., 2012. Genetics of *Borrelia burgdorferi*. Annu. Rev. Genet. 46, 515–536.

Chen, Z., Liu, Q., Liu, J.Q., Xu, B.L., Lv, S., 2013. Tick-borne pathogens and associated co-infections in ticks collected from domestic animals in Central China. Parasites Vectors. 7, 237.

Feng, X., 2016. The prospects on camel milk industry development in China. Modern Economic Information 351 (In Chinese).

Guo, J., 2009. Origin and evolution on camel. [D] Inner Mongolia Agricultural University.

Guy, E.C., Stanek, G., 1991. Detection of *Borrelia burgdorferi* in patients with Lyme disease by the polymerase chain reaction. J of Clin. Pathology 44, 610–611.

Helmy, N., 2000. Seasonal abundance of *Ornithodoros* (O.) *savignyi* and prevalence of infection with Borrelia spirochetes in Egypt. J. Egypt Soc. Parasitology 2, 607–619.

Hou, X., Gene, Z., Hao, Q., Zhang, Y., Wan, K., 2010. Rats, the primary reservoir hosts of *Borrelia burgdorferi*, in six representative provinces, China. Chinese J of Zoonoses 11, 1034–1036 (In Chinese).

Keesing, F., Brunner, J., Duerr, S., Killilea, M., Logiudice, K., Schmidt, K., Vuong, H., Ostfeld, R.S., 2009. Hosts as ecological traps for the vector of Lyme disease. Proc. Biol. Sci. 276, 3911–3919.

L, A., 1903. Sur la spirillose des bovidés. CR Acad. Sci. Paris 939, 591.

Li, X., 2009. Research progress on animal of Lyme disease. Vet Orientation 10, 43–46 (In Chinese).

Li, Y., Yang, J., Chen, Z., Qin, G., Li, Y., Li, Q., Liu, J., Liu, Z., Guan, G., Yin, H., Luo, J., Zhang, L., 2015. *Anaplasma* infection of Bactrian camels (*Camelus bactrianus*) and ticks in Xinjiang, China. Parasites Vectors. 8 (1).

Liu, Z.J., Hua, M.T., Shi, S.Z., Yang, Y.S., Luo, Y.Q., Gong, Z.W., 2000. Studies on relation between Lyme disease infection and human, livestock, rodents. J of Med. Pest. Control 6, 298–301 (In Chinese).

Liu, R., Wen, Z., Wang, J., Ge, J., Chen, H., Bu, Z., 2015. Absence of Middle East respiratory syndrome coronavirus in Bactrian camels in the West Inner Mongolia Autonomous Region of China: surveillance study results from July 2015. Emerg. Microbes. Infect. 4, e73.

Luo, X.P., 2012. Study on the epidemiology of onchocerciasis and the rDNA-ITS sequences of pathogen in camels. Inner Mongolia Agri. Univesity 6, 53 (In Chinese).

Mathiesen Jr., D.A., Kolbert, O.J., Tullson, C.P., Johnson, E.D., 1997. Genetic heterogeneity of *Borrelia burgdorferi* in the United States. J. Infect. Dis. 1, 98–107.

Ni, X., Jia, N., Jiang, B., Sun, T., Zheng, Y., Huo, Q., Liu, K., Ma, L., Zhao, Q., Yang, H., Wang, X., Jiang, J., Cao, W., 2014. Lyme borreliosis caused by diverse genospecies of *Borrelia burgdorferi* sensu lato in northeastern China. Clin. Microbiol. Infect. 20 (8), 808–814.

Niu, Q., Yin, H., Luo, J., 2009. Progress on Lyme disease in China. Prev. Vet. Med. 30, 5 (In Chinese).

Postic, D., Assous, M.V., Grimont, P.A., Baranton, G., 1994. Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of rrf (5S)-rrl (23S) intergenic spacer amplicons. Int. J. Syst. Bacteriol. 4, 743–752.

Postic, D., Ras, N.M., Lane, R.S., Hendson, M., Baranton, G., 1998. Expanded diversity among Californian borrelia isolates and description of *Borrelia bissettii* sp. nov. (formerly Borrelia group DN127). J. Clin. Microbiol. 12, 3497–3504.

Smith, R.D., Miranpuri, G.S., Adams, J.H., Ahrens, E.H., 1985. *Borrelia theileri*: isolation from ticks (*Boophilus microplus*) and tick-borne transmission between splenectomized calves. Am. J. Vet. Res. 46, 1396–1398.

Takada, N., Masuzawa, T., Ishiguro, F., Fujita, H., Kudeken, M., Mitani, H., Fukunaga, M., Tsuchiya, K., Yano, Y., Ma, X.H., 2001. Lyme disease *Borrelia* spp. in ticks and rodents from northwestern China. Appl. Environ. Microbiol. 67, 5161–5165.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolu-tionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.

Trees, A.J., 1978. The transmission of *Borrelia theileri* by *Boophilus annulatus* (say, 1821). Trop. Anim. Health Prod. 10, 93–94.

Vollmer, S.A., Bormane, A., Dinnis, R.E., Seelig, F., Dobson, A.D.M., Aanensen, D.M., James, M.C., Donaghy, M., Randolph, S.E., Feil, E.J., Kurtenbach, K., Margos, G., 2011. Host migration impacts on the phylogeography of Lyme borreliosis spirochaete species in Europe. Environ. Microbiol. 13, 184–192.

Wan, K., Zhang, Z., Zhang, J., Dou, G., Wang, H., Hou, X., Zhu, G., 1998. Preliminary in-vestigation on Lyme disease in animals in 20 provinces, cities and autonomous regions of China. Chin J Vector Biol. Control. 52–56 (In Chinese).

Wang, M., Wang, Y.H., Meng, P., Ye, Q., Zhang, D.L., 2013. Toxoplasma gondii infection in Bactrian camel (*Camelus bactrianus*) in China. Vet. Parasitol. 192, 288–289.

Wang, Y., Mu, L., Zhang, K., Yang, M., Zhang, L., Du, J., Liu, Z., Li, Y., Lu, W., Chen, C., Wang, Y., Chen, R., Xu, J., Yuan, L., Zhang, W., Zuo, W., Shao, R., 2015. A broad-range survey of ticks from livestock in northern Xinjiang: changes in tick distribution and the isolation of *Borrelia burgdorferi* sensu stricto. Parasites Vectors 8, 449.

Wodecka, B., 2007. Significance of red deer (*Cervus elaphus*) in the ecology of *Borrelia burg-dorferi* sensu lato. Wiad. Parazytol. 3, 231–237.

Wodecka, B., Leonska, A., Skotarczak, B., 2010. A comparative analysis of molecular markers for the detection and identification of Borrelia spirochaetes in Ixodes ricinus. J. Med. Microbiol. 59, 309–314.

Yang, L., Yang, X., Liu, Z., Zhang, L., Zhang, W., 2004. Investigation of *Parabronema skrjabini* disease of camels in Inner Mongolia region. J of Inner Mongolia Agri. University. 25, 43–45 (In Chinese).

Yang, F., Wang, Z., Wang, C., Baiyinchahan, 2008. LAMP for detection of trypanosoma evansi in the camels. Chinese J of Animal Infect. Dis. 16, 23–25 (In Chinese).

Yu, P., Liu, Z., Niu, Q., Yang, J., Yin, H., 2016. Research progress on Lyme disease. J of Anhui Agri. Sci. 35, 160–163 (In Chinese).

Zhai, B., Niu, Q., Yang, J., Liu, Z., Liu, J., Yin, H., Zeng, Q., 2017. Identification and molecular survey of *Borrelia burgdorferi* sensu lato in sika deer (*Cervus nippon*) from Jilin Province, North-Eastern China. Acta Trop. 166, 54–57.

Zhang, L., Wang, Y., Chen, C., Li, Y., Zhang, K., Du, J., Zhang, Y., Che, Z., 2014. Isolation of *Borrelia burgdorferi* in ixodes from four counties, in North Xinjiang. Chin J Epidemiol. 35, 262–265 (In Chinese).