Tick Distribution and Detection of Babesia and Theileria Species in the East and South regions of Kazakhstan

Chunli Sang

Department of Basic Medicine, School of Medicine, Shihezi University, Shihezi, Xinjiang Uygur Autonomous Region 832002, People's Republic of China

Meihua Yang

Department of Forestry, School of Agriculture, Shihezi University, Shihezi, Xinjiang, Uygur Autonomous Region 832000, People's Republic of China

Bin Xu

National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, WHO Collaborating Center for Tropical Diseases, Key Laboratory of Parasitology and Vector Biology of the Chinese Ministry of Health, Shanghai 200025, China

Guangyuan Liu

State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Science, Xujiaping 1#, Lanzhou, Gansu 730046, People's Republic of China

Yicheng Yang

School of Medicine, Shihezi University, Xinjiang Uygur Autonomous Region 832002, People's Republic of China

Bolatkhan Makhatov

Department of Anatomy, Physiology and Biochemistry, Kazakh National Agrarian University, Almaty, Kazakhstan.

Wurelihazi Hazihan

School of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang Uygur Autonomous Region 832000, People's Republic of China

Sándor Hornok

Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary

Yuan-Zhi Wang (✉ wangyuanzhi621@126.com)

Shihezi University

Research

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Abstract

Background: Piroplasmosis, caused by *Theileria* and *Babesia* species, is an economically important tick-borne disease worldwide. However, little is known about the presence of *Babesia* spp. and *Theileria* spp. in ticks from different host species in Kazakhstan.

Method: We collected adult ticks at 26 sampling sites in 16 districts in the East and South regions of Kazakhstan during 2016 - 2019. Tick species were identified according to morphological and molecular characteristics. Two fragments of the 18S ribosomal RNA (18S rRNA) were used to determine the species of piroplasms. The genotype characteristics of *Babesia caballi* and *Theileria equi* were analyzed further.

Results: A total of 6107 adult ticks were collected, including 2920 *Dermacentor marginatus*, 1442 *Argas persicus*, 903 *Hyalomma asiaticum*, 464 *Hyalomma scupense*, 225 *Hyalomma anatolicum*, 122 *Rhipicephalus turanicus*, 16 *Haemaphysalis erinacei* and 15 *Dermacentor reticulatus*. In these ticks five species of piroplasms, i.e. *Babesia occultans*, *Babesia caballi*, *Theileria ovis*, *Theileria annulata* and *Theileria equi*, were detected. *Theileria equi* and *Babesia caballi* belonged to their E and A genotype, respectively.

Conclusions: Apart from *Dermacentor marginatus*, three *Hyalomma* species predominate among ticks infesting herbivorous livestock in East and South regions of Kazakhstan. 16S ribosomal DNA (16S rDNA) phylogenetic analysis showed that several tick species in Kazakhstan, as exemplified by *D. marginatus* and *Ha. erinacei*, were clustered together with conspecific ticks reported from China. *Babesia occultans* was found for the first time in *D. marginatus* in Kazakhstan. Altogether five piroplasm species were detected, indicating that enhanced control measures are necessary to prevent piroplasm infection of domestic animals in this region.

Background

Kazakhstan, located in Central Asia, covers 2.72 million square kilometers and borders five countries including China, the Russian Federation, Kyrgyzstan, Uzbekistan and Turkmenistan. Five border oblasts in the East and South regions of Kazakhstan, namely Kyzylorda, South Kazakhstan, Jambyl, Almaty and East Kazakhstan, cover about one-third of Kazakhstan, with an east-west span of approximately 2,000 kilometers, and border Kyrgyzstan to the South and Xinjiang Uygur Autonomous Region (XUAR, northwestern China) to the East.

At least thirteen tick species have been reported from Kazakhstan, including *Hyalomma asiaticum*, *Hyalomma anatolicum*, *Hyalomma scupense*, *Hyalomma marginatum*, *Haemaphysalis punctata*, *Haemaphysalis sulcata*, *Dermacentor daghestanicus*, *Dermacentor marginatus*, *Dermacentor niveus*, *Dermacentor reticulatus*, *Ixodes persulcatus*, *Rhipicephalus turanicus*, *Rhipicephalus pumilio* and *Rhipicephalus (Boophilus) calcaratus* [1-4]. Unfortunately, there have been few comprehensive reports regarding the distribution and abundance of ticks in Kazakhstan, especially in the East and South regions.

Historically, tick-borne diseases have always represented important public health issues in Central Asia. Among the others, tick-borne encephalitis, rickettsial diseases, Q fever and Crimean Congo hemorrhagic fever are known to occur in Kazakhstan [3-6]. Piroplasms (Apicomplexa: Piroplasmida) are tick-borne parasitic protozoa, most of which belong to the genera *Theileria* and *Babesia* [7]. A number of these parasites are highly pathogenic for cattle, horses, dogs, sheep, camels and in some cases, even to humans, as they may cause high fever, lethargy, anorexia, peripheral edema, petechiation of the mucous membranes, hemolysis, tachycardia and pigmenturia [8, 9]. Bovine theileriosis caused by *Theileria annulata* and equine piroplasmosis caused by *Theileria equi* were reported in Kazakhstan [10-12]. However, the detection of *Babesia* and *Theileria* species in ticks from other livestock and wildlife species is still poorly known in this country, especially in East and South regions.

In the present study, the distribution and abundance of tick species were investigated in the East and South regions of Kazakhstan. These ticks were also screened for the presence of *Babesia* and *Theileria* DNA. In addition, genotype characteristics of *T. equi* and *Babesia caballi* in ticks of horses were evaluated.

Methods

Tick sampling and morphological identification

From March to May during 2016 - 2019, contemporaneously with the peak activities of adult ticks in Kazakhstan, ticks were collected from their hosts and in the environment at 26 sampling sites in East Kazakhstan (8), Almaty (9), Jambyl (2), Southern Kazakhstan (6) and Kyzylorda (1). Off-host ticks were collected by the dragging-flagging and artificial trapping methods [13, 14]. Engorged ticks were sampled over the entire body of each animal including cattle, horses, sheep, camels, shepherd dogs, hedgehogs and chickens [15]. Data of all specimens were recorded, including date and location of collection, host species (if relevant) and individual number. All ticks were identified morphologically according to previous reports [16, 17].

DNA extraction and molecular identification of ticks

After detailed morphological analysis, the genomic DNA was extracted from 209 ticks individually (2 to 40 individuals per tick species representing each sampling site) with the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). These DNA extracts were used for molecular taxonomic analysis of the *16S rDNA* gene [18].

Molecular and phylogenetic analyses of *Babesia* and *Theileria* species

The presence of *Babesia* and *Theileria* species was screened with a PCR amplifying two fragments (487bp and 438bp) of the *18S* ribosomal RNA (*18S rRNA*) gene, using the primers BJ1/BN2 [19] and PIRO-A/B [20]. Sequence-confirmed *Babesia occultans* and *Theileria ovis* DNA amplified in our laboratory and double distilled water were used as positive and negative controls, respectively [21]. In addition,

samples positive for *B. caballi* and *T. equi* were evaluated further by amplifying longer the *18S rRNA* gene fragments (1,380 bp for *B. caballi* and 1470 bp for *T. equi*). Nucleotide sequences of the primers used for the identification of piroplasm and tick species were shown in Appendix Table S1. PCR products were purified using the TIANGel Midi Purification Kit (TIANGEN, Beijing, China) and cloned into the pGEM-T Easy vector and subjected to sequencing. The sequences obtained here were compared to others deposited in GenBank with the BLASTn programme (<http://www.ncbi.nlm.nih.gov/BLAST/>). Phylogenetic trees were constructed using the Maximum Likelihood method with MEGA 7.0 software [22].

Results

A total of 6107 adult ticks, belonging to five genera and eight species (2920 *D. marginatus*, 1442 *A. persicus*, 903 *Hy. asiaticum*, 464 *Hy. scupense*, 225 *Hy. anatolicum*, 122 *Rh. turanicus*, 16 *Haemaphysalis erinacei* and 15 *D. reticulatus*), were collected in five border oblasts of Kazakhstan (Fig. 1 and Appendix Table S2). *D. marginatus* (64.29%, 2920/4542) was the most abundant species on the herbivorous mammals, followed by *Hy. asiaticum* (17.53%, 796/4542), *Hy. scupense* (10.22%, 464/4542), *Hy. anatolicum* (4.95%, 225/4542). *A. persicus* was collected from chicken and *Ha. erinacei* from hedgehog.

Piroplasms were molecularly detected in 698 out of 6107 sampled ticks by amplifying 487 bp and 438 bp *18S rRNA* fragments. Among these, 1.43% (10/698) and 3.44% (24/698) of samples were positive for *Babesia* and *Theileria* species, respectively. Based on sequence comparisons, two *Babesia* species (*B. occultans* and *B. caballi*) and three *Theileria* species (*T. ovis*, *T. annulata* and *T. equi*) were identified (Table 1, Fig. 2 and Appendix Table S3). Phylogenetic analyses using *18S rRNA* fragments revealed that *T. equi* belonged to genotype E and *B. caballi* to genotype A (Fig. 3A and 3B).

Table 1 Detection of *Babesia* spp. and *Theileria* spp. in ticks sampled from the East and South regions of Kazakhstan.

Oblast	District	Tick species	Origination	Number	<i>Babesia/ Theileria</i> species, No. positive (%)
Almaty	Balkhash County (BH)	<i>Hyalommaaiaticum</i>	Off host	47	<i>Babesia caballi</i> , 1 (2.13%)
		<i>Haemaphysaliserinacei</i>	Hedgehog	16	0
	Shagan City (SN)	<i>Rhipicephalusturanicus</i>	Goat	24	<i>Theileriaannulata</i> , 5 (33.33%)
	Usharal County (UR)	<i>Hyalomma scupense</i>	Cattle	14	0
	Ushtobe County (UT)	<i>Dermacentor marginatus</i>	Horse	22	0
	Karabulak County (KL)	<i>Dermacentormarginatus</i>	Horse	10	<i>Babesia caballi</i> , 1 (10%) <i>Theileria equi</i> , 2 (20%)
East Kazakhstan	Zaysan County (ZS)	<i>Hyalomma scupense</i>	Cattle	48	0
		<i>Dermacentormarginatus</i>	Cattle	12	0
	Shyghys qazaqstan City (SQ)	<i>Dermacentormarginatus</i>	Cattle	15	0
South Kazakhstan	Chimkent City (CK)	<i>Dermacentormarginatus</i>	Cattle	36	<i>Babesia caballi</i> , 2 (5.56%)
			Horse	24	<i>Theileria equi</i> , 6 (25%)
		<i>Argas persicus</i>	Chicken	102	0
	Saryagash County (SG)	<i>Dermacentormarginatus</i>	Cattle	159	<i>Babesia caballi</i> , 4 (2.30%) <i>Theileria annulata</i> , 4 (2.30%) <i>Babesia occultans</i> , 2 (1.45%)
		<i>Dermacentorreticulatus</i>	Horse	15	
	Kazygurt County (KG)	<i>Rhipicephalus turanicus</i>	Dog	24	<i>Theileria annulata</i> , 4 (16.67%) <i>Theileria ovis</i> , 1 (4.17%)
Zhambyl	Merki (MK)	<i>Dermacentormarginatus</i>	Cattle	20	0
	Lugovoy (LG)	<i>Hyalomma anatolicum</i>	Cattle	40	0

Kyzylorda	Aralsk County (AL)	<i>Hyalomma asiaticum</i>	Camel	70	0
Total 698					

Discussion

In the present study, eight tick species were found in five border oblasts in the East and South regions of Kazakhstan. To our knowledge, the following records had been reported previously in this area: *I. persulcatus* in East Kazakhstan, *Hy. asiaticum*, *Rh. turanicus*, *Hy. scupense*, *D. niveus* and *D. marginatus* in Kyzylorda, *Hy. asiaticum* and *Hy. scupense* in Jambyl, *Hy. anatolicum*, *Hy. asiaticum*, *Ha. punctata*, *Ha. sulcata* and *Hy. scupense* in South Kazakhstan, *Hy. asiaticum*, *Hy. scupense*, *I. persulcatus*, *Ha. punctata*, *D. marginatus* and *D. reticulatus* in Almaty region [1, 3, 23]. This implies that *D. marginatus* and *Hy. anatolicum* were detected for the first time in Jambyl, and further four species (*D. marginatus*, *D. reticulatus*, *Rh. turanicus* and *A. persicus*) in South Kazakhstan, extending our knowledge on tick species in these regions. Compared with previous studies, *D. marginatus* and *Hy. scupense* can be considered as emerging tick species in East Kazakhstan, similarly to *Rh. turanicus* in Almaty.

Southeastern Kazakhstan and Northwestern China share 1,760 km borderline. In the present study, *D. marginatus* and *Hy. asiaticum* were the most abundant tick species in five border oblasts of Kazakhstan. Interestingly, *D. marginatus* and *Hy. asiaticum* were also dominant tick species in the neighboring Xinjiang Uygur Autonomous Region (XUAR, northwestern China) [24]. Furthermore, the phylogenetic tree based on *16S rDNA* sequences showed that *D. marginatus* ticks in Almaty, Jambyl and South Kazakhstan clustered together with those from XUAR (GenBank: MF002564). In addition, *Hy. asiaticum* (MN907445) in Almaty represented the same phylogenetic group as ticks from Turkmenistan (KU13042), Turkey (MG418675) as well as XUAR of China (MF973043) (Fig. 4). *Hy. scupense*, however, showed different abundances on domestic animals in Southeastern Kazakhstan compared to Northwestern China. According to the present results, this species is widely distributed in East Kazakhstan and Almaty on cattle and goats, while it is rare in XUAR [24].

1. *B. occultans* was firstly described from *Hyalomma rufipes* in 1981 in South Africa [25]. Subsequently, it was found in engorged *rufipes*, *Hyalomma truncatum*, *Hyalomma impressum*, *Hyalomma marginatum* and *Hyalomma impeltatum* ticks in 1984 in Nigeria [26]. For a long time, the distribution of *B. occultans* was believed to be confined to sub-Saharan Africa. However, in 2017 it was shown to be present in *Dermacentor nuttalli* in XUAR [21]. Here *B. occultans* detected for the first time in *D. marginatus* ticks in Kazakhstan clustered phylogenetically with *B. occultans* (MF120939) reported from Jimunai County of XUAR (Fig. 2). Taken together, in this study five species of piroplasms, i.e. *B. occultans*, *B. caballi*, *T. ovis*, *T. annulata* and *T. equi* were detected in South Kazakhstan, suggesting that enhanced control measures are necessary to prevent piroplasm infection of domestic animals in this region.

The *18S rRNA* gene is widely used as a genetic marker for phylogenetic analyses of piroplasms [27]. Previous studies reported that *T. equi* genotype E was associated with fatal cases of equine piroplasmosis in Greece and Spain [28, 29]. Although increasing amounts of data on equine piroplasmosis had become available from Spain, Mongolia, Egypt and China [28, 30-32], no studies aimed to examine the genotype of *T. equi* and *B. caballi* in Kazakhstan. Here, phylogenetic analysis showed that *B. caballi* in Almaty and South Kazakhstan belonged to genotype A, which was also present in China (MH651221), South Africa (EU642512), Spain (AY309955) and Brazil (KY952235). At the same time, *T. equi* (MN857679 and MN857677) from South Kazakhstan clustered into genotype E of this species, together with isolates from Spain (AY534882), China (MH651213 and KF559357) and South Korea (HM229407).

Conclusions

In this study, eight tick species and five piroplasm species were found in five border oblasts in the East and South regions of Kazakhstan. *16S* ribosomal DNA phylogenetic analysis showed that several tick species from Kazakhstan, such as *D. marginatus* and *Ha. erinacei*, clustered with conspecific ticks from China. *B. occultans* was found for the first time in *D. marginatus* in Kazakhstan. Genotype E of *T. equi* and genotype A of *B. caballi* were shown to occur in the study region.

Abbreviations

18S rRNA: *18S* ribosomal RNA; *16S rDNA*: *16S* ribosomal DNA; XUAR: Xinjiang Uygur Autonomous Region.

Declaration

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Authors' contributions

CS, MY, BX and YW conceived and designed the study, and writing the manuscript. CS, GL, YY, BM and WH performed the experiments, analyzed the data. SH critically revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data supporting the conclusions of this article are included within the article. The datasets used and analyzed during the present study are available from the corresponding author upon reasonable request.

Ethics and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Basic Medicine, School of Medicine, Shihezi University, Shihezi, Xinjiang Uygur Autonomous Region 832002, People's Republic of China. ²Department of Forestry, School of Agriculture, Shihezi University, Shihezi, Xinjiang, Uygur Autonomous Region 832000, People's Republic of China. ³National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, WHO Collaborating Center for Tropical Diseases, Key Laboratory of Parasitology and Vector Biology of the Chinese Ministry of Health, Shanghai 200025, China. ⁴State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Science, Xujiaping 1#, Lanzhou, Gansu 730046, People's Republic of China. ⁵Emergency Department, Shihezi City People's Hospital, Shihezi, Xinjiang Uygur Autonomous Region 832000, People's Republic of China. ⁶Department of Anatomy, Physiology and Biochemistry, Kazakh National Agrarian University, Almaty, Kazakhstan. ⁷School of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang Uygur Autonomous Region 832000, People's Republic of China. ⁸Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary.

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Figures

Figure 2

Phylogenetic analysis of *Babesia* spp. and *Theileria* spp. in ticks collected from five border oblasts of Kazakhstan. The tree was constructed with the Maximum Likelihood method (ML; bootstrap replicates: 1000), using concatenated sequence data of the 18S rRNA gene with MEGA7. *Babesia* and *Theileria* sequences obtained in this study are indicated by solid triangle (▴) and solid square (▣), respectively.

Figure 3

Phylogenetic tree of *T. equi* and *B. caballi* genotype inferred from partial sequences of the 18S rRNA gene. A: Genotypic analysis with the sequences of *T. equi*, B: Genotypic analysis with the sequences of *B. caballi*. The tree was constructed with the Maximum Likelihood method (ML; bootstrap replicates: 1000), using concatenated sequence data of the 18S rRNA gene with MEGA7. Sequences of *T. equi* and *B. caballi* obtained in this study are indicated by solid diamond (◆) and solid triangle (▲), respectively.

Figure 4

Phylogenetic analysis of representative tick specimens: sequences of the tick species obtained in this study are indicated by solid circle (●). The tree was constructed with the Maximum Likelihood method (ML; bootstrap replicates: 1000), using sequence data of the 16S rDNA gene with MEGA7.

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