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ORIGINAL PAPER

Some aspects on tick species in Mongolia and their potential role in the transmission of equine piroplasms, *Anaplasma phagocytophilum* and *Borrelia burgdorferi* L.

Myadagsuren Narankhajid[1,2] · Chultemsuren Yeruult[2] · Agvaandaram Gurbadam[2] · Jigjav Battsetseg[3] · Stephan W. Aberle[4] · Badamdorj Bayartogtokh[5] · Anja Joachim[1] · Georg Gerhard Duscher[1]

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Abstract

Ticks are cosmopolitan vectors of numerous diseases, and detection of various pathogens in ticks can help to assess their distribution. In the current study, 528 adult ticks were collected from grazing animals or the ground in ten different Mongolian provinces. *Dermacentor nuttalli* constituted 76.1% of them and was found in all ecozones except the eastern desert. *Dermacentor marginatus* (8.3%), *Dermacentor silvarum* (1.1%) and *Ixodes persulcatus* (3.0%) were found in the northern forest areas and *Hyalomma asiaticum* (11.4%) only in the southern (semi-)desert. Of these, 359 ticks were subjected to DNA extraction and PCR was carried out to detect various pathogens. *Anaplasma* spp. was found in *D. marginatus* and *D. nuttalli* (2.5% positive each), including flagged specimen and identified as *Anaplasma phagocytophilum*. *Borrelia* spp. were found in 2.5% of the ticks (mostly in *I. persulcatus*) and identified as *Borrelia garinii*. *Babesia* spp. (40%) identified as *Babesia caballi* were detected in all five tick species including flagged *Dermacentor* spp. and *I. persulcatus*, and 3.5% of the ticks (all species except *D. silvarum*) were positive for *Theileria* spp. identified as *Theileria equi*. The piroplasms were found in all provinces. Tick-borne encephalitis virus was not detected. The results highlight the high risk of equine piroplasmosis in Mongolia, which is a concern for both the nomadic population who rely on horses for transport and for conservation of Przewalski's horses in Mongolia. In addition, zoonotic agents such as the avian *B. garinii* and *A. phagocytophilum* were also detected, outlining a high risk for exposed humans.

Keywords Anaplasmosis · Borreliosis · Piroplasmosis · TBE · Horses · Ixodid ticks

✉ Georg Gerhard Duscher
Georg.Duscher@vetmeduni.ac.at

Myadagsuren Narankhajid
narankhajid@mnums.edu.mn

Chultemsuren Yeruult
eruultch@mnums.edu.mn

Agvaandaram Gurbadam
gurbadam@nmums.edu.mn

Jigjav Battsetseg
jigjavb@gmail.com

Stephan W. Aberle
stephan.aberle@meduniwien.ac.at

Badamdorj Bayartogtokh
bayartogtokh@num.edu.mn

Anja Joachim
Anja.Joachim@vetmeduni.ac.at

[1] Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine Vienna, Veterinaerplatz 1, 1210 Wien, Austria

[2] Mongolian National University of Medical Sciences, S. Zorig Street 3, Ulaanbaatar 14210, Mongolia

[3] National Centre for Zoonotic Diseases, Ministry of Health, Songinokhairkhan 20, Ulaanbaatar 14219, Mongolia

[4] Department of Virology, Medical University of Vienna, Kinderspitalgasse 15, 1095 Vienna, Austria

[5] Department of Biology, National University of Mongolia, Zaluuchuud Avenue 1, Ulaanbaatar 14201, Mongolia

Introduction

Ticks are notorious vectors of various pathogens that cause serious and life-threatening infections in humans and animals worldwide (Estrada-Peña and Jongejan 1999; Parola and Raoult 2001; Zhang 2012; Wu et al. 2013). In many cases, screening of ticks for such pathogens with molecular tools can identify their presence in particular geographic regions. While a number of these agents primarily pose a threat to animal health, such as *Theileria* spp. and *Babesia* spp., the agents of piroplasmosis in various animals (Krause 2002; Irwin 2010; Suarez and Noh 2011; Wise et al. 2013; Beugnet and Moreau 2015; Alvarado-Rybak et al. 2016; Solano-Gallego et al. 2016; Tarav et al. 2017), can harm both animals and humans, for instance *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis (Stuen et al. 2013), *Borrelia burgdorferi* sensu lato which causes Lyme disease and is considered an emerging or spreading disease worldwide (Stone et al. 2017; Strnad et al. 2017). Tick-borne encephalitis virus (TBEV) is one of the most important causes of inflammatory disease on the central nervous system in humans in Europe and Asia (including Mongolia; Khasnatinov et al. 2010) which is also considered to be an emerging disease (Jaenson et al. 2012; Valarcher et al. 2015); it has a wildlife reservoir and also can infect dogs and cats (Duscher et al. 2014).

In central Asian countries including Mongolia, the above-mentioned tick-borne pathogens have previously been detected in ticks by DNA analysis or in mammals by direct or indirect detection methods. *Anaplasma* spp. and *Borrelia* spp. were detected in Mongolian ticks (Fomenko et al. 2009; Scholz et al. 2013; Masuzawa et al. 2014; Iwabu-Itoh et al. 2017; Javkhlan et al. 2014), livestock (Papageorgiou et al. 2012; Karnath et al. 2016; Ochirkhuu et al. 2017) and humans (Walder et al. 2006; von Fricken et al. 2018). Piroplasms were detected in ticks (Battsetseg et al. 2001, 2002; Boldbaatar et al. 2005; Tuvshintulga et al. 2015, 2016; Karnath et al. 2016), livestock (Avarzed et al. 1997; Ruegg et al. 2007; Altangerel et al. 2011, 2012; Sivakumar et al. 2012; Munkhjargal et al. 2013; Yoshinari et al. 2013) and humans (Hong et al. 2014) in Mongolia. TBEV was isolated from ticks (Frey et al. 2012) and detected in humans in Mongolia (Walder et al. 2006; Khasnatinov et al. 2010; Muto et al. 2015). However, most of these studies were limited in sample size, the pathogens detected or geographical coverage and most of them did not consider multiple infections in ticks.

Mongolia is known for its pastoral animal husbandry where the local herders raise more than 61 million livestock (National Statistical Office of Mongolia 2017; http://www.en.nso.mn), which constitutes a major resource for their economy. Since animal husbandry is based on a nomadic lifestyle, domestic and wild animals are frequently in both direct and indirect contact because they share the same pastures and water sources, and cross-infection can easily occur. Prevention of the spread of infections, including zoonotic diseases, is therefore highly important in this country.

The goal of the present preliminary investigation was to evaluate the frequency of tick species from different areas of Mongolia and of the pathogens they harbour to provide data for a disease risk assessment for several important tick-borne diseases (anaplasmosis, borreliosis, equine piroplasmosis and tick-borne encephalitis).

Materials and methods

Study area

Mongolia is a landlocked country in eastern and central Asia with around 3.1 million inhabitants. It borders Russia to the north and the People's Republic of China to the south, east and west. The land area of Mongolia (1.564.116 km^2 or 603,909 square miles; www.wikipedia.com) is divided into 21 provinces (*aimags*) with 334 districts. The country contains very little arable land, as much of its area is covered by steppes, with mountains to the north and west and the Gobi Desert to the south. Approximately 30% of Mongolia's population is nomadic, and pastoral herders spend prolonged periods with their livestock. This close proximity to animals and working outdoors increases the potential for exposure to ticks and the pathogens they harbour for humans, which can directly impact the lives of Mongolians by causing illness and indirectly through economic losses incurred from illness in livestock (von Fricken et al. 2018). Distributions, abundance, habitat preference of ticks in various regions and ecosystems of Mongolia and their vertebrate host spectrum have been described previously (Dash et al. 1988; Danchinova et al. 2007, 2012).

Sample collection

A total of 528 adult ticks were collected from domestic animals and by flagging from the environment in ten different provinces (Bayankhongor, Gobi-Altai, Huvsgul, Selenge, Bulgan, Dornogobi, Hovd, Zavkhan, Tuv and Arkhangai provinces) covering the western, northern and southern parts of Mongolia (Fig. 1, Tables 1 and 2). Specifically, horses were sampled in four provinces (Gobi-Altai, Bulgan, Hovd and Tuv). The ticks were identified according to standard taxonomic keys (Schulze and Schlottke 1930; Khishigsuren 2002).

DNA extraction, PCR amplification and sequence analysis

Adult ticks were air-dried on microscope slides for 5 min to allow excess ethanol to evaporate and dissected lengthwise

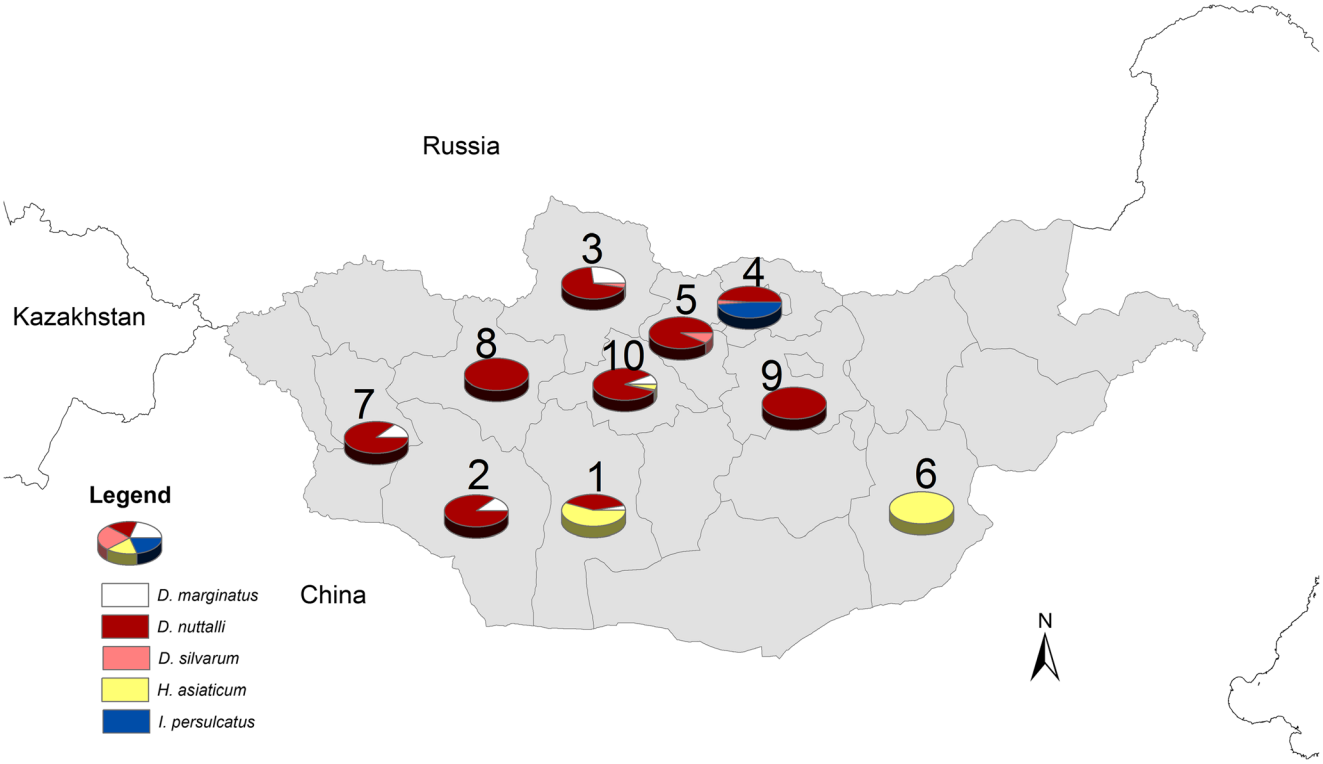

Fig. 1 Ticks collected from 10 provinces in Mongolia. 1 = Bayankhongor ($n = 84$), 2 = Gobi-Altai ($n = 82$), 3 = Huvsgul ($n = 60$), 4 = Selenge ($n = 33$), 5 = Bulgan ($n = 24$), 6 = Dornogobi ($n = 13$), 7 = Hovd ($n = 72$), 8 = Zavkhan ($n = 40$), 9 = Tuv ($n = 40$) and 10 = Arkhangai ($n = 80$) provinces. Tick species are indicated with pie sections of different colours

Table 1 Distribution of tick-borne pathogens in different provinces of Mongolia

Province	Ticks collected/examined (*n*)	Number (%) of PCR-positive samples			
		Anaplasma	Borrelia	Babesia	Theileria
1. Bayankhongor	84/64	0	2	27	0
2. Gobi-Altai	82/61	2[a]	2	27	0
3. Huvsgul	60/49	7[b]	0	31	0
4. Selenge	33/11	0	3[c]	5	1
5. Bulgan	24/17	0	0	12	2[f]
6. Dornogobi	13/8	0	0	3[d]	1
7. Khovd	72/45	0	1	20	8[g]
8. Zavkhan	40/20	0	0	6	0
9. Tuv	40/27	0	1	3	0
10. Arkhangai	80/58	0	0	11[e]	1
Total	528/360	9 (2.5%)	9 (2.5%)	145 (40.3%)	13 (3.6%)

Sequence confirmations (in parentheses: number of specimen):

[a] *A. phagocytophilum* G variant from *D. nuttalli*, feeding on sheep (1) and goat (1)

[b] *A. phagocytophilum* G variant from questing *D. marginatum* (1), *A. phagocytophilum* A variant from questing *D. nuttalli* (6)

[c] *B. garinii* from questing *I. persulcatus* (2)

[d] *Ba. caballi* from *H. asiaticum* attached to camel (1)

[e] *Ba. caballi* from *D. nuttalli* attached to goat (1)

[f] *T. equi* from *D. nuttalli* feeding on sheep (2)

[g] *T. equi* from *D. nuttalli* feeding on horses (4)

Table 2 Patterns of infestation by adult ticks and tick-borne pathogens for different vertebrate hosts and the environment in the study provinces

Host (source)	Tick species	Number of collected ticks	DNA extraction positive ticks	Infected ticks	Detected pathogens infecting ticks by PCR			
					Anaplasma	*Borrelia*	*Babesia*	*Theileria*
Camel	*D. marginatus*	7	2	2	0	0	2	0
	D. nuttalli	41	30	10	0	1	9	0
	H. asiaticum	16	15	8	0	1	6	1
	Subtotal	64	47	20	0	2	17	1
Horse	*D. marginatus*	3	3	4	0	0	2	2
	D. nuttalli	40	25	23	0	1	15	7
	D. silvarum	2	0	0	0	0	0	0
	Subtotal	45	28	27	0	1	17	9
Sheep	*D. marginatus*	5	4	1	0	0	1	0
	D. nuttalli	85	58	29	1	1	26	1
	H. asiaticum	5	5	2	0	0	2	0
	Subtotal	95	67	32	1	1	29	1
Goat	*D. marginatus*	10	9	4	0	0	4	0
	D. nuttalli	113	87	31	1	2	28	0
	H. asiaticum	33	23	8	0	0	8	0
	Subtotal	156	119	43	1	2	40	0
Cattle	*D. marginatus*	3	1	0	0	0	0	0
	D. nuttalli	62	36	6	0	0	5	1
	D. silvarum	1	0	0	0	0	0	0
	H. asiaticum	6	1	1	0	0	1	0
	Subtotal	72	38	7	0	0	6	1
Human	*D. nuttalli*	2	1	0	0	0	0	0
	Subtotal	2	1	0	0	0	0	0
Field	*D. marginatus*	16	16	8	1	0	7	0
	D. nuttalli	59	35	31	6	1	24	0
	D. silvarum	3	2	1	0	0	1	0
	I. persulcatus	16	7	7	0	2	4	1
	Subtotal	94	60	47	7	3	36	1
Total		528	360	176 (48.9%)	9 (2.5%)	9 (2.5%)	145 (40.3%)	13 (3.6%)
Sequences obtained					9 (100%)	2 (22.2%)	36 (24.8%)	9 (69.2%)

with sterile scalpel blades. Half of the tick was used for DNA isolation with the DNeasy tissue kit (QIAGEN GmbH, Germany) according to the manufacturer's instructions. Isolated DNA was eluted in 100 µl AE buffer (QIAGEN GmbH) and aliquots were frozen immediately at − 80 °C for subsequent analysis.

For molecular identification of tick-borne pathogens, PCR was performed on a Biometra T-Gradient thermocycler using primers and cycling conditions as described in Table 3. Each reaction (25 µl) contained 5 µl of template DNA, 5 µl of 5× Green GoTaq® reaction buffer (Promega, Mannheim, Germany) 200 µM of each dNTP and 0.75 U GoTaq® DNA polymerase (Promega). A negative control with distilled water instead of template DNA and positive controls (DNA of *Anaplasma*, *Borrelia*, *Theileria* or *Babesia*) were included in each run.

PCR products were separated using an ethidium bromide-stained 2% agarose gel (2:1 sieve agarose; Biozym, Austria) in Tris-acetate-EDTA buffer and visualised under UV light. A DNA molecular weight marker (Promega) was used to estimate product sizes. The positive PCR products were purified (QIAquick PCR Purification kit, QIAGEN GmbH, Germany) according to the manufacturer's instructions, and the purified products were sent to Microsynth AG for sequence determination (Sanger Sequencing Division, Switzerland).

Sequences were assembled and edited with AlignPlus® software (version 4.1). Sequences obtained in the present study were compared with the corresponding sequences deposited in GenBank® by using the BLAST software of the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Data analysis

Data were analysed using SPSS 14.0 software. Correlations were calculated using Pearson's and Spearman's rank correlation coefficients.

Table 3 Primers used for PCR for the detection and identification of tick-borne pathogens in ticks from Mongolia

Target genus of species	Target gene	Forward primer (5′–3′) Reverse primer (3′–5′)	Annealing temp. (°C)	No. of cycles	Expected amplicon size (bp]	References
Anaplasma spp.	groESL	AGACGAAATTGCACAAGTT AGCCTTTGCTTTCTTCAAC	64	55	128	Polin et al. 2004
Borrelia spp.	rpoB	GATGATATTGACCATTTAGG TTCAGGGGTTTCAATAGGAC	63	40	369	Lee et al. 2000
Babesia spp.	18S rRNA	GACTAGGGATTGGAGGTC GAATAATTCACCGGATCACTC	53	30	650	Blaschitz et al. 2008
Theileria spp.	18S rRNA	TGACACAGGGAGGTAGTGA TCAGCCTTGCGACCATACT	60	35	1590	Sawczuk et al. 2008

Nucleotide sequence accession numbers The 18S rRNA gene sequences of *Babesia caballi* are available at GenBank® [GenBank: JQ288735 and JQ288736]. The 18S rRNA gene sequences of *Theileria equi* is available at GenBank® [GenBank: JQ657703]. The RNA polymerase beta subunit gene sequence of *Borrelia garinii* is available at GenBank® [GenBank: JQ657704]. The sequences for *A. phagocytophilum* were too short for submission.

Results

Ticks

In total, 528 adult ticks (244 females and 284 males) collected from ten different provinces located in various ecological areas were attributed to five species from three genera of different abundancies (Fig. 1 and Table 4). The distribution of species varied in different ecozones. *Dermacentor nuttalli* (76.1%) was found in all ecological zones (according to the Köppen-Geiger classification; https://en.wikipedia.org/wiki/K%C3%B6ppen_climate_classification) except the most eastern desert (Dornogobi province). It was the only species collected in the grassland areas of Tuv and Arkhangai. *Dermacentor marginatus* (8.3%) was collected from taiga-forest and forest-steppe of the northern, central and western areas; it was most frequent in the northern Huvsgul province. *Dermacentor silvarum* (1.1%) was only found in the forest-steppe of the northern Bulgan province. *Ixodes persulcatus* (3.0%) was collected only in the northern taiga-forest and forest-steppe of Selenge province. *Hyalomma asiaticum* (11.4%) was found in the desert and semi-desert ecosystems in the south and south-east (Dornogobi, where only eight specimen of this species were collected) and was the dominating species of these areas (Fig. 1; Table 1). All tick species except *H. asiaticum* could be flagged from the ground; this species was removed from camel and ruminants only (Table 2).

DNA analysis

DNA extraction was successful in only 360 (68.2%) of the ticks, most probably due to prolonged and unsuitable storage and transportation conditions. Similarly, sequences could only be obtained in completeness for all *A. phagocytophilum*-positive samples, while rpoB (*Borrelia* species determination) and 18S rDNA (for piroplasms) sequence determinations were only partially successful.

Table 4 Distribution of infections by tick species, including multiple infections

	D. marginatus	*D. nuttalli*	*D. silvarum*	*I. persulcatus*	*H. asiaticum*	All species
N ticks (% of all specimen)	44 (8.3)	402 (76.1)	6 (1.1)	16 (3.0)	60 (11.4)	528
N ticks with DNA extraction (%)	35 (79.5)	272 (67.6)	2 (33.3)	7 (43.8)	44 (73.3)	360 (68.2)
Infected ticks (%)	54.3	48.0	50.0	100	43.2	48.9
Anaplasma positive in (%)	2.9	3.0	0.0	0.0	0.0	2.5
Borrelia positive (%)	0.0	2.2	0.0	28.6	2.3	2.5
Babesia positive (%)	45.7	39.5	50.0	57.1	38.6	40.3
Theileria positive (%)	5.7	3.3	0.0	14.3	2.3	3.6
Anaplasma + *Babesia* positive (%)	0.0	3.7	0.0	0.0	0.0	2.8
Borrelia + *Babesia* positive (%)	0.0	1.1	0.0	28.6	2.3	1.7
Theileria + *Babesia* positive (%)	5.7	2.2	0.0	14.3	2.3	2.8

Nine ticks, collected in the Gobi-Altai and Huvsgul provinces, were positive for *Anaplasma* spp. by PCR (Table 1). *D. nuttalli* and *D. marginatus* collected from sheep, goats, or the field (*D. marginatus*, *D. nuttalli*) were positive (Table 2). The 126-bp region of the *A. phagocytophilum* heat shock operon *groESL* gene was amplified from each positive specimen and was 100% homologous to *A. phagocytophilum* sequences from Russia, Spain, France and Slovenia [GenBank® accession numbers HQ629910, HM057232, EU860091 and EU381150]. Six amplicons (all derived from field-collected *D. nuttalli* from the Huvsgul province) could be identified as groSEL-A variants, three (two *D. nuttalli* from the Gobi-Altai region collected from sheep and goat, respectively, and one from a questing *D. marginatus* from Huvsgul) were groSEL-G variants.

The nine *Borrelia*-positive *I. persulcatus* and *D. nuttalli* were collected from goats, sheep, camel, horses or the field (Table 2) in five different provinces with prevalence rates of 2.2–3.7% for Hovd, Bayankhongor, Gobi-Altai and Tuv and 27.3% in Selenge (Table 1). For two of the positive isolates (from questing *I. persulcatus* in Selenge), *rpoB* gene sequences could be obtained and revealed > 99% similarity with *B. garinii* [GenBank® accession numbers AF164225, AF164221 and AF164224] with the highest level of similarity to *B. garinii* IP89 strain from Korea [GenBank® accession number AF164225].

Babesia spp. were detected in 145 ticks of all five species in all provinces with prevalences from 11.1% in Tuv to 70.6% in Bulgan province (Tables 1 and 2). Positive ticks were collected from grazing animals or the field (questing *Dermacentor* spp. and *I. persulcatus*; Table 2). Only two 18S rRNA gene sequences could be obtained, one from *H. asiaticum* from (camel in Dorngobi) and one from *D. nuttalli* (a goat in Arkhangai). Both were 98–100% similar to *Ba. caballi* from Africa and Spain [GenBank® accession numbers Z15104, E888904, Z15105, DQ287954, AY150062 and AY150063].

Thirteen ticks from five provinces (prevalences 1.7–17.8%; Table 1) collected from sheep, horses, cattle, camels and the field were positive for *Theileria* (Tables 2 and 4). In particular, *D. marginatus* and *D. nuttalli* from horses and one questing *I. persulcatus* were positive for *Theileria*. The five amplicons obtained 18S rRNA gene sequence (1590 bp) were > 99% similar with *T. equi* [GenBank® accession numbers HM229408, HM229407, DQ287951 and AY534882]. The sequences were all derived from *D. nuttalli* (three from horses in Hovd and one from a horse and one from a sheep in Bulgan). No TBEV nucleic acids could be detected by PCR in any of the examined ticks.

Multiple infections were diagnosed in 26 ticks of all species except *D. silvarum*. Ten *D. nuttalli* were positive for *Anaplasma* and *Babesia*, six ticks from three different species were positive for *Borrelia* and *Babesia*, and four different species for *Theileria* and *Babesia* (Table 4). Differences in the prevalence rates of mixed infections were not observed between the study provinces (details not shown).

Discussion

Distribution of ticks and their possible roles as vectors

In the present study, 528 adult ticks from ten Mongolia's provinces were determined to species level and 360 of them were analysed for different tick-borne pathogens. The most abundant species was *D. nuttalli* with constituted 76.1% of the ticks and was also the most widely distributed. In the present study, *D. nuttalli* was found in all ecological zones except the most eastern desert area (Dornogobi province) as reported earlier (Dash et al. 1988; Danchinova et al. 2007, 2012). Of the 271 *D. nuttalli* from which DNA could be extracted, 46.1% were infected with *Anaplasma*, *Borrelia* or piroplasms and the finding of these pathogens (with the exception of *Theileria*) in questing specimen of *D. nuttalli* highlights the possible role of this tick species as a vector of both human and animal pathogens as it was also the only species found on all animals as well as humans. Specifically, *D. nuttalli* is a well-known vector for equine piroplasms (Battsetseg et al. 2002, 2001; Scoles and Ueti 2015); however, in this study, sequences of *Ba. caballi* and *T. equi* could only be confirmed in feeding ticks (six *D. nuttalli* and one *H. asiaticum*) and the exact percentage of these species in the ticks could not be determined.

The second most common tick species was *H. asiaticum* which had a rather restricted range in (semi-)arid areas, in accordance with earlier works (Dash et al. 1988) and a recent study by Boldbaatar et al. (2017); 43.2% of the examined specimens were positive for *Borrelia* or piroplasms. A number of *Hyalomma* spp. have been described as vectors for pirolasms of equids (see Scoles and Ueti 2015, for review) and bovine *Theileria annulata* in the Middle East (Mazlum 1968) but *H. asiaticum* was not known to transmit piroplasms so far. The presence of *Ba. caballi* in one *H. asiaticum* specimen feeding on a camel does not sufficiently support this assumption since vector competence cannot be determined in feeding ticks. As *Hyalomma* specimen are difficult to flag (Duscher, unpublished) no ticks of this species could be collected from the environment and the definitive vector capacity still remains to be determined.

D. marginatus, the ornate sheep tick, is a tick species with a wide distribution in Eurasia (Rubel et al. 2016). This species is common in the forest-steppe and steppe pasturelands of central, northern, western and southwestern areas of Mongolia (Dash et al. 1988), but of low abundance in the present study, with 54.3% of the examined specimens being positive for *Anaplasma* spp., *Borrelia* spp. or piroplasms. It can transmit a number of pathogens and has already been inferred as vector of equine piroplasms (Scoles and Ueti 2015) but final determination if this is still lacking. Besides *D. nuttalli*, *D. marginatus* was the only tick species infected with *Anaplasma* which includes questing ticks so the vector role of these two species must be assumed. In the present study, only *A. phagocytophilum* could be detected by PCR and the role of these ticks as vectors for other *Anaplasma*

spp. present in Mongolian livestock (Ochirkhuu et al. 2017) could not be determined here.

I. persulcatus, the taiga tick is abundantly distributed in the Siberian taiga-forest and forest-steppe areas of northcentral, northwestern and northeastern parts of Mongolia (Danchinova et al. 2007). It constituted only 3.0% of the collected ticks, but can nevertheless be considered a major vector of various pathogens. It not only transmits *A. phagocytophilum* in the northern areas of Mongolia (Javkhlan et al. 2014; Masuzawa et al. 2014; Karnath et al. 2016), but also borreliae (Kurtenbach et al. 2006; Fomenko et al. 2009) including the bird-associated *B. garinii* identified in the present study. It is the most important vector of TBEV in Mongolia (Baasandavga et al. 2017). In the present study, TBEV could not be detected in ticks, which is not surprising as other methods of surveillance are generally considered to be more sensitive (Stefanoff et al. 2013; Imhof et al. 2015). The vectorial role of *I. persulcatus* in Mongolia also includes the zoonotic microorganisms *Babesia* sp. "venatorum" (known also as Babesia sp. EU1) and *Candidatus* Neoehrlichia mikurensis (Karnath et al. 2016), so it must be considered as significant for human health (see also Jaenson et al. 2016, for review on the vector capacity of *I. persulcatus*), despite of the small number of ticks collected and its presumably restricted geographical location in Mongolia, as described previously (Filippova 1985; Boldbaatar et al. 2017). At a global scale, *I. persulcatus* is widely distributed and is considered to be spreading westwards (Jaenson et al. 2016).

According to Danchinova et al. (2007), *D. silvarum* occurs primarily in central and northern Mongolia in low abundance. *D. silvarum* was only sporadically detected in the present study in a single province; one of the two analysed specimens was positive for *Babesia*, as were the other, more abundant representatives of this genus. This does not allow for assessment of the role of this species as vector for the pathogens in question.

Tick-borne pathogens

Babesia and *Theileria* were the most frequently detected pathogens in this study. *T. equi* and *Ba. caballi* both infect the red blood cells of horses and cause similar systemic diseases with high mortalities of up to 50% (de Waal 1992; de Waal and van Herden 1994; Wise et al. 2013; Beugnet and Moreau 2015). Due to the wide distribution of available tick vectors with high rates of piroplasms detected by PCR in Mongolia, equine piroplasmosis remains a significant threat to the health of horses also in this part of the world. *Ba. caballi* is the most commonly reported agent of horse babesiosis in the central, eastern and western regions of Mongolia (Avarzed et al. 1997; Xuan et al. 1998; Battsetseg et al. 2002; Boldbaatar et al. 2005). It was found in questing ticks of all detected tick species in all provinces and its prevalence was higher in ticks from horses (64.2%) than in ticks from other grazing animals (15.8–51.7%). *T. equi* was only detected in a single questing specimen of *I. persulcatus*, a tick that has not previously been reported to be a vector for this protozoal pathogen (de Waal and van Herde 1994; Scoles and Ueti 2015). It was also detected in *Dermacentor* spp. from horses as well as from *H. asiaticum* and *D. nuttalli* feeding on non-permissive hosts so a vector role can be assumed for these species. Equine husbandry in Mongolia has remained a pastoral nomadic system, which includes seasonal migrations and rotations of migration routes. Movement through endemic areas may contribute to infestation of horses with *Dermacentor* spp. and infections with *T. equi* and *Ba. caballi* (Battsetseg et al. 2001).

The prevalence rates for the two species in horses vary in different studies. Seropositivity was reported to be 51.6–84.5% for *Ba. caballi* and 19.6–88.2% for *T. equi* with mixed infections in 10.4%. PCR detected *Ba. caballi* in up to 20.0% of examined horses and *T. equi* in up to 92.7%; mixed infections were < 3% (Avarzed et al. 1997; Xuan et al. 1998; Sloboda et al. 2011; Munkhjargal et al. 2013; Tarav et al. 2017). Differences in the detection rates in horses are presumed to be a result of different transmission dynamics of the two parasites (despite concomitant detection of both parasite species in questing ticks; ref. this study and Battsetseg et al. 2001, 2002). In addition, parasitaemia for *T. equi* is considered to be much longer than that of *Ba. caballi*, and *Ba. caballi*-infected horses may therefore be seropositive without being infected anymore (Ruegg et al. 2006). Reintroduced Przewalski's horses (takhis) showed 84% positivity for *T. equi*, which is a concern since this parasite is responsible for mortalities of up to 19% in young wild horses (Tarav et al. 2017).

In addition to animal pathogens such as equine piroplasms, zoonotic agents such as *A. phagocytophilum* and *B. garinii* were found in considerable rates in the examined ticks, indicating that not only animals but also humans bear a high risk of infection when exposed to ticks which is common for nomads in Mongolia (Papageorgiou et al. 2012; von Fricken et al. 2018).

Anaplasma phagocytophilum has previously been detected in *I. persulcatus* as described above; it is a cosmopolitan pathogen and the causative agent of granulocytic ehrlichiosis worldwide. Its main vectors are *Ixodes* spp. (Stuen et al. 2013) but it has also been found in *Haemaphysalis* spp., *D. nuttalli* and *D. silvarum* in China (Wei et al. 2016). In the present study, *Anaplasma* was not detected in *I. persulcatus*, most likely due to the small sample size for this tick. Sequence analysis of the *groESL* genes of nine samples in this study revealed that six sequences were 100% identical to the *A. phagocytophilum groESL-A* variant mostly found in wild ungulates (Liz et al. 2002; Petrovec et al. 2003), three were 100% identical to the *groESL*-G variant primarily found in horses (Sumner et al. 1997; Loewenich et al. 2003), dogs (Smrdel et al. 2009) and humans (Sumner et al. 1997).

Lyme borreliae are widespread in Russia (Korenberg 1995; Nataliya et al. 2008) and China, with *B. garinii* as the main genotype, distributed mainly in its northern part (Wu et al.

2013). This species was also detected in two questing *I. persulcatus* from Selenge.

Coinfections were detected mainly with *Babesia* spp. in line with the relative detection rates for the pathogens in different ticks, as described previously for Asian *I. persulcatus* (Masuzawa et al. 2014; Karnath et al. 2016; Boldbaatar et al. 2017). Overall, 26/176 (14.8%) positive ticks were coinfected with two pathogens. An increasing number of studies report that ticks and their vertebrate hosts often harbour multiple infections, suggesting that this might be the rule rather than the exception (Belongia 2002; Swanson et al. 2006; Ginsberg 2008; Raileanu et al. 2017), and hosts infected with several pathogens may show more severe symptoms of diseases (Krause 2002; Pańczuk et al. 2016; Kaewmongkol et al. 2017).

Since Babesia-positive samples were quite frequent and products were not cloned to detect multiple infections, it is possible that infections with more than one piroplasmid species remained undetected and the number of multiple infections was underestimated. More detailed studies on piroplasmid infections should be carried out in future works to decipher any hidden or masked pathogens of this taxon.

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Authors' contributions AJ and GD conceived and designed the study and critically revised the manuscript. MN, GD, BB and SWA performed the experiments, analysed the data and drafted and revised the manuscript. CY, AG and JB provided samples of ticks from field survey. All authors have approved the final manuscript.

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