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SUPPLEMENT ARTICLE

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Molecular detection of tick-borne pathogens in bovine blood and ticks from Khentii, Mongolia

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Abstract

Recent studies reported the detection of DNA from tick-borne pathogens (TBPs) of veterinary relevance such as *Anaplasma marginale*, *Babesia bigemina*, *Babesia bovis* and *Theileria orientalis* in bovine blood samples from Mongolia. These findings were unexpected, as the known tick vectors of these pathogens are not known to occur in Mongolia. We therefore conducted a study in May and June 2013 in six districts of Khentii province where DNA of the said TBPs was previously found. Ticks collected from the vegetation and rodents, as well as blood samples from cattle, were screened for the presence of TBPs by reverse line blot (RLB) hybridization. Tick larvae collected from rodents were pooled. A total of 310 adult ticks were collected from the vegetation, and 249 tick larvae were collected from 24 rodents. Adult ticks (n = 2,318) and blood samples were collected from 481 heads of cattle. All adult ticks were identified as *Dermacentor nuttalli*. DNA from *Rickettsia raoultii* (252/310; 81.3%), an uncharacterized *Anaplasma* species preliminary named *Anaplasma* sp. Mongolia (26/310; 8.4%), *Candidatus* Midichloria sp. (18/310; 5.8%), *Theileria equi* (16/310; 5.2%), *Babesia caballi* (5/310; 1.6%), *T. orientalis* (1/310; 0.3%), *Borrelia afzelii* (1/310; 0.3%) and *Candidatus* Neoehrlichia mikurensis (1/310; 0.3%) was detected in ticks collected from the vegetation. DNA of *R. raoultii* (27/28; 96.4%) and *Midichloria* sp. (2/28; 7.1%) was detected in the pooled tick larvae. *Anaplasma* sp. Mongolia, a species related to *Anaplasma ovis* based on a multi-locus analysis, was also detected in 153/481 (31.8%) of the bovine blood samples. DNA of *B. bovis*, *B. bigemina* and *A. marginale* was not detected in the ticks or bovine blood samples from Khentii district.

KEYWORDS
Dermacentor nuttalli, molecular detection, Mongolia, tick-borne pathogens

1 | INTRODUCTION

Mongolia is the second-largest landlocked country in the world, located in Central Asia between Russia and China. The country has a pronounced continental climate with warm summers and very cold winters. The traditional lifestyle of its inhabitants is nomadic, and although changing environmental and socio-economic conditions have impacted Mongolian pastoralism, livestock husbandry still plays

a pivotal role in the economy and culture of Mongolia (Batsukh et al., 2013). The occurrence of infectious livestock diseases in Mongolia could therefore threaten the livelihoods of many. This is why recent reports on the detection of DNA from the causal agents of bovine anaplasmosis (*Anaplasma marginale*), bovine babesiosis (*Babesia bigemina* and *B. bovis*) and *Theileria orientalis* in Mongolian cattle were cause for concern (AbouLaila, Yokoyama, & Igarashi, 2010; Altangerel et al., 2011, 2012; Sivakumar et al., 2012; Ybanez et al., 2013), as these pathogens are of great veterinary and economic relevance and had never been reported from this part of the world before. Bovine anaplasmosis is usually characterized by fever, weight and production loss, abortion, and death, whereas bovine babesiosis may result in fever, haemolytic anaemia, haemoglobinuria and in severe cases, death (Bock, Jackson, de Vos, & Jorgensen, 2004; Kocan, de la Fuente, Blouin, Coetzee, & Ewing, 2010; Watts, Playford, & Hickey, 2016). The introduction of these pathogens in a naïve population would typically lead to outbreaks of clinical anaplasmosis and babesiosis (Hue et al., 2013; Kocan et al., 2010). In areas where the tick infection rate with *A. marginale*, *B. bigemina* and/or *B. bovis* is high and calves are challenged at a young age, the inverse age immunity described for these pathogens may lead to endemic stability, a state with low clinical disease incidence (Jonsson, Bock, Jorgensen, Morton, & Stear, 2012). *Theileria orientalis* has long been considered to be a benign parasite, but the emergence of a novel *T. orientalis* genotype (Ikeda) is associated with outbreaks of clinical theileriosis in Australia, Japan and New Zealand (Watts et al., 2016).

All of the above-mentioned pathogens were reportedly detected by PCR in a set of 300 bovine blood samples, or a subset thereof, collected in three provinces located in the west (Uvs), centre (Uvurkhangai) and east (Khentii) of Mongolia in May 2010 (Altangerel et al., 2011). Assays used included a conventional PCR for the detection of *T. orientalis* and nested PCRs for the detection of *A. marginale*, *B. bigemina* and *B. bovis* (Altangerel et al., 2011; Sivakumar et al., 2012; Ybanez et al., 2013). A serosurvey of cattle samples collected between 2014 and 2016 in Mongolia showed a seroprevalence of 50.6% (90/178) for *B. bigemina* and of 25.3% (45/178) for *B. bovis* in cattle from Khentii province (Battsetseg et al., 2018).

Although mechanical transmission of *A. marginale* by biting flies or blood-contaminated fomites has been well-documented (Kocan et al., 2010), biological transmission of *A. marginale*, *B. bigemina* and *B. bovis* is mainly associated with one-host *Rhipicephalus* ticks, which occur in subtropical and tropical regions of the world (Roy, Estrada-Pena, Krucken, Rehman, & Nijhof, 2018). The transmission of *T. orientalis* in Asia and Australasia is mainly associated with *Haemaphysalis longicornis* (Uilenberg, Perie, Spanjer, & Franssen, 1985). Interestingly, none of the tick species previously associated with the transmission of these pathogens are known to occur in Mongolia, where seven tick species are known to infest cattle: *D. nuttalli*, *D. silvarum*, *D. marginatus* (previously reported under the synonym *D. daghestanicus* (Estrada-Pena & Estrada-Pena, 1991)), *Haemaphysalis pospelovashtromae*, *Hyalomma asiaticum*, *Hy. dromedarii*, *Ixodes persulcatus* and *Rhipicephalus pumilio* (Arthur, 1960; Dash, Bjamba, & Splisteser, 1989; Walker, Keirans, & Horak, 2000).

To investigate the prevalence of *A. marginale*, *B. bigemina*, *B. bovis* and *T. orientalis* in Mongolian cattle and elucidate local transmission routes, a cross-sectional survey was performed to collect ticks and blood samples from cattle in six districts of Khentii province. Ticks were also collected from the vegetation and rodents, to examine if circulating *Babesia* strains are transmitted transovarially in local tick species. Khentii province was selected as the most suitable study site since it was reported to be the region with the highest overall infection rates of the above-mentioned pathogens. The collected samples were subsequently screened for the presence of TBPs using a reverse line blot hybridization assay (RLB; Nijhof et al., 2007).

2 | MATERIALS AND METHODS

2.1 | Sample collection

The field survey was conducted in May and June 2013 in six districts (soums) in Khentii province: Tsenkhermandal, Dadal, Jargaltkhaan, Binder, Delgerkhaan and Bajan-Adarga. DNA of *A. marginale*, *B. bigemina*, *B. bovis* and *T. orientalis* was previously detected in bovine blood samples collected in the first four districts (Altangerel et al., 2012; Sivakumar et al., 2012; Ybanez et al., 2013). Samples were collected from ten herds in each district, with the exception of Tsenkhermandal, where samples were collected from 11 herds. The number of cattle living in these districts was thought to vary between approx. 5,000 heads of cattle in Delgerkhaan to ~20,000 heads of cattle in Binder. With an expected prevalence of at least 5% based on the studies cited above, a confidence level of 95% and assuming a perfect test, it was calculated that at least 58 animals should be sampled per district in order to detect the above-mentioned pathogens (Fosgate, 2009). Since it cannot be assumed that the RLB is a perfect test, the sample size was increased to 80 animals per district. Consequentially, blood samples and ticks were collected from eight one- to three-year-old animals from each herd. As only six animals could be sampled in one herd in Delgerkhaan, the remaining two samples were collected from the 11th herd in Tsenkhermandal for logistical reasons. Selection of the animals was based on convenience sampling, that is the first eight animals that became available during the visit to the herd. Blood was collected from the *Vena jugularis* in ethylenediaminetetraacetic acid (EDTA) containing vacutainer tubes (BD Biosciences, Franklin Lakes, NJ, USA) and initially chilled in a cooler with frozen cold packs. During sampling, the body of the animals was inspected for the presence of ticks. If present, ticks were collected and stored in 70% ethanol. A number of ticks collected from cows were stored in vodka with an alcohol content of 38% (ERUUL vodka, APU Company) in instances where 70% ethanol was not available. These ticks were identified to the species level, but DNA was not extracted for further downstream analysis. At the end of each sampling day, 125 µl of EDTA blood was spotted on Flinders Technology Associates (FTA) Classic cards (FTA cards, Whatman Biosciences) and allowed to air-dry over night at room temperature. The remaining EDTA blood was subsequently stored at −20°C. Ticks were also collected *ad hoc* from the vegetation near the herds by

flagging and by manual removal from the yurts of the herdsman. In Jargaltkhaan and Bajan-Adarga, rodents were trapped in June 2013 using Sherman live animal traps. All the observed ticks were collected from the rodents and stored in 70% ethanol. The rodents were subsequently released. All procedures involving animals were conducted by trained veterinary staff and in accordance with regulations of the Mongolian Institute of Veterinary Medicine, Ulaanbaatar, Mongolia.

2.2 | Tick identification

Ticks were identified at the Institute for Parasitology and Tropical Veterinary Medicine of the Freie Universität Berlin using a stereomicroscope and several identification keys (Arthur, 1960, 1962; Sondermann, 1993; Tyron, 1983). To confirm the morphological identification of the tick larvae, DNA was extracted from eight randomly selected larval ticks using the Nucleospin Tissue Kit according to the manufacturer's instructions (Macherey-Nagel). DNA was subsequently amplified by PCR using primers Metastriata ITS2-F (5′-AGGACACACTGAGCACTGATTC-3′) and Metastriata ITS2-R (5′-ACTGCGAAGCACTTRGACCG-3′). PCRs were performed in a 25 µl volume with 5 µl of 5 × Phusion HF buffer (Thermo Fisher Scientific), 2.5 µl of 2 mM dNTPs, 1 µl of 10 pM of each forward and reverse primer, 0.25 µl of Phusion Hot Start II High-Fidelity DNA polymerase (Thermo Fisher Scientific), 2.5 µl of genomic DNA template and 12.75 µl of deionized water. PCR conditions were 98°C for 30 s, followed by 40 cycles of 98°C for 7 s, 60°C for 10 s and 72°C for 20 s and a final extension phase at 72°C for 5 min in a C1000 Touch thermocycler (Bio-Rad Laboratories). Following gel electrophoresis, amplicons were purified using the Zymoclean gel DNA recovery kit (Zymo Research Corporation) following manufacturer's instructions and sent for Sanger sequencing by LGC Genomics (Berlin, Germany).

2.3 | DNA extraction

DNA was extracted from 200 µl of the EDTA blood using the Nucleospin Blood kit (Macherey-Nagel) following the manufacturer's instructions. In 32 cases where EDTA samples were lost during transport, DNA was extracted from FTA cards following a previously published protocol (Hailemariam, Ahmed, Clausen, & Nijhof, 2017). Ticks were washed twice in distilled water and air-dried prior to homogenization in 1.5 Eppendorf tubes with disposable pestles and subsequent DNA extraction using the Nucleospin Tissue 8 kit (Macherey-Nagel) following the manufacturer's instructions. The quality and quantity of the extracted DNA samples were measured using a Take3 plate in an Epoch Microplate Spectrophotometer (BioTek®). All DNA extracts were stored at −20°C.

2.4 | Molecular detection of tick-borne pathogens

A PCR targeting the 5S-23S rRNA intergenic spacer region of *Borrelia burgdorferi* sensu lato was conducted with primers 5SCB and 23SN2 (Schouls, Van De Pol, Rijpkema, & Schot, 1999). The 18S rRNA gene of *Babesia* and *Theileria* species and the 16S rRNA gene of *Anaplasma*, *Ehrlichia*, *Rickettsia* and *Midichloria* species were amplified using primers RLB-F2/RLB-R2 (Gubbels et al., 1999) and Ehr-F2/Ehr-R (Hailemariam, Ahmed et al., 2017). The PCRs were performed in a 25 µL reaction volume consisting of 1.25 U Maxima Hot Start polymerase (Thermo Fisher Scientific), 2.5 µl Maxima Hot Start Buffer, 2.5 µl $MgCl_2$ (25 mM), 200 µM of each dNTP, 1 µl of each primer (10 pmol/µl) and 2.5 µl of the template DNA. PCR cycle parameters for the amplification of the 16S and 18S rRNA genes included an initial denaturation at 95°C for 30 s, followed by 10 cycles of 95°C for 20 s, 67°C for 30 s and 72°C for 30 s, with lowering of the annealing step after every second cycle by 2 °C. The reaction was then followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 58 °C for 30 s and extension at 72°C for 30 s. For the amplification of the *Borrelia* interspacer region, all annealing temperatures were 8 °C lower. All PCRs were performed in a C1000 thermal cycler (Bio-Rad Laboratories). Positive controls used included *A. marginale*, *B. bovis* and *T. orientalis* DNA from previous studies (Hailemariam, Ahmed et al., 2017; Roy, Krucken et al., 2018). The RLB assay was performed as previously described (Hailemariam, Ahmed et al., 2017). For the detection of the previously uncharacterized *Anaplasma* species, the probe *Anaplasma* sp. Mongolia (5′-CCA CCA AGG CGG TGA TCT GT-3′) containing a C12-aminolinker was used.

For the phylogenetic analysis of a previously uncharacterized *Anaplasma* species, a larger fragment of the 16S rRNA gene was amplified from bovine blood DNA as previously described (Rehman, Conraths, Sauter-Louis, Krucken, & Nijhof, 2018). In addition, a 1446-nt fragment of the *groEL* and a 632-nt fragment of the *msp5* gene were amplified from the same sample using primer combinations AMgroES-111F1/AMgroES1557R1 and AM-49F1/AM595R1, respectively (Ybanez et al., 2013). The PCR mastermix for amplification of both *msp5* and *groEL* genes was identical to the Maxima mastermix described above. Amplification conditions were an initial denaturation at 95°C for 4 min, followed by 35 cycles at 95°C for 20 s, 58°C for 30 s and 72°C for 30 s (msp5) or 100 s (groEL) and a final extension step at 72°C for 7 min. PCR products (5 µl) were analysed by gel electrophoresis on 1.5% (W/V) agarose gels stained with GR Green (Labgene Scientific). Gel images were obtained using a G:box imaging system (Syngene). Samples were sequenced by LGC Genomics (Berlin) following purification of the PCR products using the Zymoclean gel DNA recovery kit (Zymo Research) according to the manufacturer's protocol.

2.5 | Phylogenetic analysis

A maximum-likelihood multi-locus phylogenetic analysis was performed based on the 16S rRNA and the *groEL* DNA sequences as previously described (Roy, Krucken et al., 2018). The sequences used are presented in Table S1.

2.6 | Statistical analysis

Confidence intervals (95%) for frequencies were calculated as Wilson score intervals using the propCI 0.3-3 package in R 3.5.1 (Scherer, 2018).

3 | RESULTS

3.1 | Ticks

In total, 310 adult ticks were collected from the vegetation, of which 169 were male and 141 female. All ticks were identified as *D. nuttalli*. The number of ticks that could be collected ranged from 4 in Tsenkhermandal to 118 in Delgerkhaan (Table S2). Ticks were also collected from 24 trapped rodents. In Jargaltkhaan, 193 tick larvae were collected from 14 rodents, and in Bajan-Adarga, 56 tick larvae were collected from 9 rodents. One trapped rodent from Bajan-Adarga was not infested with ticks. Of these 249 larvae, 49 were damaged and could not be properly identified. The ITS2 gene from eight of the remaining 200 larvae, which were all identified as *Dermacentor* spp. but could not be morphologically identified to the species level, was 100% identical to an ITS2 gene sequence of *D. nuttalli* (GenBank Accession Number KF241874) from Siberia. A total of 2,318 adult *D. nuttalli* ticks were collected from the bodies of the 481 heads of cattle from which blood was collected (Table S3). Details regarding the infestation rate per animal were not recorded.

3.2 | Pathogens

Screening of the DNA extracted from ticks collected from the vegetation by RLB revealed the presence of DNA from *Rickettsia raoultii* (252/310; 81.3%), a previously uncharacterized *Anaplasma* sp. which was preliminary named *Anaplasma* sp. Mongolia (26/310; 8.4%), *Candidatus* Midichloria sp. (18/310; 5.8%), *Theileria equi* (16/310; 5.2%), *Babesia caballi* (5/310; 1.6%), *T. orientalis* (1/310; 0.3%), *Borrelia afzelii* (1/310; 0.3%) and *Candidatus* Neoehrlichia mikurensis (1/310; 0.3%; Table 1).

DNA was also extracted from pooled larvae; each pool represented the larvae collected from a single rodent. Nearly, all pools (96.4%, 27/28) tested positive for *R. raoultii* and two pools (7.1%) tested positive for the *Candidatus* Midichloria genus probe in the RLB (Table 1).

Anaplasma sp. Mongolia was detected in 31.8% (153/481) of the bovine blood samples (Table 1). The *msp5* sequences (MK583951) showed 91.8% identity (580/632) to the *msp5* sequence of *Anaplasma ovis* (CP015994). Its 16S rRNA sequence (MK575506) showed highest sequence identity (99.8%, 1390/1393 nt) to the sequence of an *Anaplasma* species isolated from the blood of cattle and yaks in Mongolia (GenBank Accession Number LC194132) and from *D. nuttalli* ticks (99.6%, 479/481 nt; Accession Number MG461482). The *groEL* gene sequence (MK583950) was nearly identical (99.6%, 502/504 nt) to that of an *Anaplasma* species (JQ735903) previously detected in the blood of cattle in Undurkhaan, Mongolia (Ybanez et al., 2013). A multi-locus analysis based on the 16S rRNA and *groEL* gene sequences of *Anaplasma* sp. Mongolia showed that it clustered with *A. ovis* isolates from China and South Africa (Figure 1). DNA of *A. marginale*, *B. bigemina* and *B. bovis* was not detected in any of the samples. None of the interviewed herdsmen (n = 61) reported mortality or clinical signs associated with bovine anaplasmosis, babesiosis or theileriosis.

TABLE 1 Frequency of tick-borne pathogens in ticks and cattle blood samples

	Number	*Theileria equi*	*Theileria orientalis*	*Babesia caballi*	*Babesia bovis/ bigemina*	*Rickettsia raoultii*	*Anaplasma* sp. Mongolia[a]	*Anaplasma marginale*	*Candidatus* Neoehrlichia mikurensis	*Candidatus* Midichloria sp.	*Borrelia afzelii*
Cattle blood samples	481	0	0	0	0	0	153	0	0	0	0
		0%	0%	0%	0%	0%	31.8%	0%	0%	0%	0%
Adult ticks	310	18	1	5	0	252	26	0	1	18	1
		5.8%	0.3%	1.6%	0%	81.3%	8.4%	0%	0.3%	5.8%	0.3%
Larval tick pools	28	0	0	0	0	27	0	0	0	2	0
		0%	0%	0%	0%	96.4%	0%	0%	0%	7.1%	0%

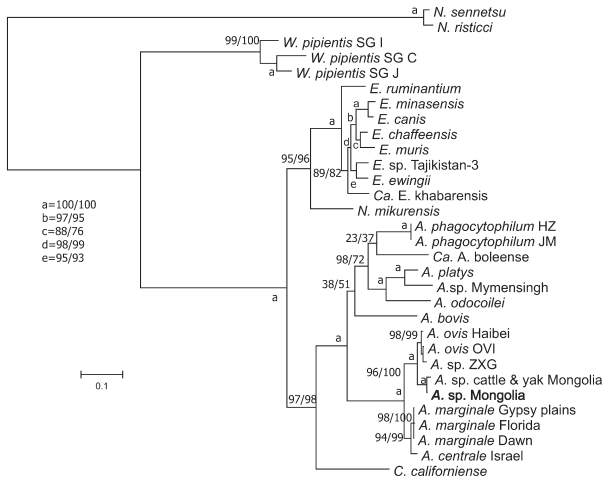

FIGURE 1 Maximum-likelihood phylogenetic analysis of Anaplasmataceae using a multi-locus analysis based on 16S rRNA and *groEL* gene sequences. *Neorickettsia risticci* and *Neorickettsia sennetsu* were included as outgroup. Numbers before and after the slash represent node support values obtained by the bootstrapping (1,000 replicates) and Shimodaira–Hasegawa likelihood ratio test, respectively. The scale bar represents 0.1 substitutions per site

4 | DISCUSSION

Reports on the molecular detection of the causal agents of bovine anaplasmosis and babesiosis in Mongolia, outside of their known distribution range, prompted us to conduct a field study in Khentii province to determine the prevalence of these pathogens and examine their local epidemiology. In previous reports, Khentii province was noted to be the region with the highest overall infection rates: 8.0% (17/212) for *A. marginale*, 10.6% (23/218) for *B. bovis* and 8.1% (15/185) for *B. bigemina* (Altangerel et al., 2012; Sivakumar et al., 2012; Ybanez et al., 2013). Another source reports an infection rate of up to 95.8% (23/24) for *B. bovis* in bovine blood samples collected before 2010 in Mongolia, but details on the exact origin of these samples were not provided (AbouLaila et al., 2010). Other TBPs previously reported to occur in ruminants from Khentii were *T. orientalis*, with an infection rate of 42.5% (90/212) in the same set of samples from May 2010 (Altangerel et al., 2011) and *Anaplasma ovis*, which was detected in blood samples collected from sheep (7.4%, 2/27) and goats (13.3%, 2/15) in Khentii (Enkhtaivan et al., 2019).

The tick fauna of Mongolia comprises 14 species (Dash et al., 1989), with *D. nuttalli* being the most common tick collected from domestic animals (Altangerel et al., 2011; Enkhtaivan et al., 2019; Narankhajid et al., 2018). The activity period of ticks in Mongolia generally lasts from March to June for adults and from May to September for juveniles, with a peak in July and August (Dash, 1988). Since May and June are the months in which the activity of all life stages overlaps, these months were selected for performing the field study to collect ticks. Blood samples collected in May and June, that is 2–3 months after the start of the tick season, would also be likely to contain circulating TBPs, since animals that survive an acute infection of *A. marginale*, *B. bigemina* or *B. bovis* develop persistent infections that last for at least several months (Bock et al., 2004; Kocan, de la Fuente, Blouin, & Garcia-Garcia, 2004). However, none of the 481 blood samples, 310 adult ticks collected from the vegetation or pools of tick larvae collected from rodents tested positive for *A. marginale*, *B. bigemina* and *B. bovis* by RLB. This is in line with results of a previous survey in which 928 blood samples collected in central Mongolia from large and small ruminants were examined by PCR for the presence of *Anaplasma* species and in which *A. marginale* could not be detected either (Ochirkhuu, Konnai, Odbileg, Murata, & Ohashi, 2017). The use of convenience sampling or differences in the detection limits of the tests used might form possible explanations for these findings, since parasitemias in asymptomatic carriers can be low and fluctuate over time (Calder et al., 1996). The detection limit of the RLB was previously found to correspond to a parasitemia of 0.00006% for *B. bovis* and of 0.00002% for *A. marginale* (Hailemariam, Ahmed et al., 2017), which is indeed less sensitive than the reported detection limit of 0.000000001% for the *B. bovis SBP2* nPCR and of 2 copies/PCR for the *A. marginale msp5* nPCR, which were previously used to detect *B. bovis* and *A. marginale* in Mongolia (AbouLaila et al., 2010; Ybanez et al., 2013).

The presence of *T. orientalis* in Khentii was confirmed in a single questing tick, but this pathogen was not detected in any of the bovine blood samples. This is also in contrast with a previous report in which 42.5% (90/212) of the sampled cattle from Khentii was found to be PCR-positive for *T. orientalis* (Altangerel et al., 2011). Although the RLB was shown to be suitable for the detection of *T. orientalis* in bovine blood samples even in laboratory experiments conducted in parallel with the present study (Gubbels et al., 1999; Hailemariam, Kruckenet al., 2017; Roy, Krucken et al., 2018), it cannot be excluded that differences in sampling methodology or detection limits between the used assays caused the different results. The transmission cycles and veterinary relevance of potential *A. marginale*, *B. bigemina*, *B. bovis* and *T. orientalis* infections in Mongolian cattle thus remain to be elucidated.

Other TBPs that were detected by RLB included the uncharacterized *Anaplasma* sp. Mongolia in 31.8% (153/481) of the bovine blood samples and 8.4% (26/310) of the adult *D. nuttalli* ticks collected from the vegetation. This species is closely related to *A. ovis* and was recently found in other studies as well, where it is sometimes reported as *A. ovis* (Moore et al., 2018; Ochirkhuu et al., 2017; Ybanez et al., 2013). The vector for this bacterium might be *D. nuttalli*, as it was detected in questing adult ticks and there is some evidence of transovarial transmission of this *Anaplasma* species in *D. nuttalli* (Moore et al., 2018). Further studies are however required to confirm this and determine the pathogenicity of *Anaplasma* sp. Mongolia for livestock and humans.

The most common TBP detected in our study was *R. raoultii*: 81.3% of the questing adult ticks and 96.4% of the tick pools tested positive. This corroborates previous findings that reported a similar prevalence rate of 82% (147/179) in questing *D. nuttalli* ticks from Mongolia (Speck et al., 2012) and can also be explained by a high level of transovarial transmission (Moore et al., 2018). *Rickettsia raoultii* is a spotted fever group (SFG) rickettsia that is

predominantly found in *Dermacentor* species in Europe and Asia and has been associated with a syndrome characterized by scalp eschars and neck lymphadenopathy in humans (Liu et al., 2016; Parola et al., 2013). Reports from China also describe the isolation of *R. raoultii* from patients presented with a febrile illness and localized rashes (Jia et al., 2014; Li et al., 2018). A recent study on the seroprevalence of SFG *Rickettsia* showed a considerable exposure to SFG Rickettsia in Mongolian herders (19.5%, 73/374) and livestock (20.4%, 478/2342), to which *R. raoultii* may have contributed (von Fricken et al., 2018). Other SFG Rickettsia reported to occur in Mongolia are *R. sibirica* and *Candidatus* R. tarasevichiae (Boldbaatar et al., 2017; Lewin, Bouyer, Walker, & Musher, 2003).

DNA of two other zoonotic pathogens, *B. afzelii* and *Candidatus* N. mikurensis, was detected in questing *D. nuttalli* ticks. *Borrelia afzelii* has previously been detected in *I. persulcatus* ticks from Mongolia (Masuzawa et al., 2014; Sabitova et al., 2018) and other species of the *B. burgdorferi* sensu lato complex are known to occur in Mongolia as well, but little is known about the clinical impact of Lyme borreliosis in the country (Walder et al., 2006). *Candidatus* N. mikurensis is regularly found in *Ixodes* ticks and rodents in Europe and Asia and was recognized as a human pathogen causing a febrile illness in 2010 (Kawahara et al., 2004; Portillo, Santibanez, Palomar, Santibanez, & Oteo, 2018; Welinder-Olsson, Kjellin, Vaht, Jacobsson, & Wenneras, 2010). In Mongolia, this bacterium was detected in *I. persulcatus* ticks (Karnath et al., 2016), and although it has also been detected in questing *D. reticulatus* ticks in Germany (Krucken et al., 2013), this is the first report of its detection in *D. nuttalli*. The role of this tick species in the epidemiology of neoehrlichiosis is not clear. We also detected *Candidatus* Midichloria sp. for the first time in *D. nuttalli* adults and larvae. *Candidatus* Midichloria sp. are symbionts of ticks which are also secreted with tick saliva, causing an immune response in infested humans and animals (Bazzocchi et al., 2013; Cafiso et al., 2019). It is most frequently found in *Ixodes* ticks, but has been reported in other tick genera, including *Dermacentor*, as well (Dergousoff & Chilton, 2011).

Equine piroplasmosis is widespread in Mongolia (Boldbaatar et al., 2005; Sloboda et al., 2011), and the detection of its causal agents, *B. caballi* and *T. equi* in a number of questing *D. nuttalli* ticks, is consistent with previous publications (Battsetseg et al., 2001, 2002; Narankhajid et al., 2018).

This study revealed the presence of multiple pathogens of medical and veterinary relevance in ticks and bovine blood samples collected in Khentii province, Mongolia. Additional studies are required to study their clinical impact and epidemiology. In contrast to previous reports, we could not detect *A. marginale*, *B. bigemina* and *B. bovis* in local cattle and ticks. The origin and epidemiology of these TBP in the Mongolian cattle population remain enigmatic.

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ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. At the time this study was conducted, an ethical review committee was not yet established in Mongolia. The Guidelines of the American Society of Mammalogists for the use of wild mammals in research for the trapping of rodents and the guidelines for the collection of blood from the German Society for Laboratory Animal Science (GVSOLAS) were adhered to instead.

CONFLICT OF INTEREST

The authors have no conflict of interests concerning the work reported in this manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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