Molecular detection of *Anaplasma ovis* in small ruminants and ixodid ticks from Mongolia

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ABSTRACT

Anaplasma ovis is a tick-borne obligate intracellular rickettsial bacterium that causes anaplasmosis in domestic and wild small ruminants. Sheep and goats, whose combined population is approximately 48.5-million in Mongolia, play a vital role in the country's economy. In this study, we conducted an epidemiological survey of *A. ovis* in sheep and goats from 19 of 21 provinces in Mongolia. Additionally, DNA samples extracted from unfed ticks collected in 11 Mongolian provinces were also screened for *A. ovis*. Of 1179 and 871 blood DNA samples from sheep and goats, 813 (69.0%) and 621 (71.3%), respectively, were positive for *A. ovis* when screened by a PCR assay based on major surface protein 4 gene (*msp4*). On a per province basis, *A. ovis* infection rates ranged from 7.4%–93.3% and 13.3%–100% in sheep and goats, respectively. Subsequently, DNA samples prepared from 721 unfed ticks, including *Dermacentor nuttalli* (n = 378)*, Ixodes persulcatus* (n = 95)*, Haemaphysalis pospelo-vashtromae* (n = 120)*, and Hyalomma asiaticum* (n = 128), were screened for *A. ovis* using the same PCR assay. Although nine *D. nuttalli* were *A. ovis*-positive, all other tick DNA samples were negative. In addition to reporting *A. ovis* in sheep and goats from all over Mongolia, this study identified *D. nuttalli* as a potential transmission vector of *A. ovis* in Mongolia. The present data highlight the importance of monitoring Mongolian sheep and goats for possible episodes of clinical anaplasmosis and controlling *D. nuttalli* throughout the country.

1. Introduction

Anaplasma ovis, a Gram-negative bacterium, belongs to genus *Anaplasma*, family Anaplasmataceae, and order Rickettsiales, and infects domestic and wild small ruminants [1,2]. *Anaplasma ovis* is transmitted by ticks and infects host erythrocytes, where asexual reproduction occurs [3]. In sheep and goats, *A. ovis* infection might be characterised by mild-to-severe clinical disease [2,4–6]. The disease development is often predisposed by co-infection with other pathogens and stress induced by various factors, such as hot climate and transportation [2,4,5]. The clinical signs of anaplasmosis caused by *A. ovis* in sheep and goats include fever, anaemia, jaundice, abortion, and production losses [7]. Therefore, control of *A. ovis* infection is vital for

successful sheep and goat farming, and tick control is an integral part of any *A. ovis* control strategy, as tick control prevents *A. ovis* transmission from ticks to ruminants and vice versa.

Mongolia is an agricultural country, and the livestock industry plays a critical role in its national economy. However, growth of this industry has often been undermined by several factors, including infectious diseases [8]. Various species of tick-borne blood pathogens, including those of *Anaplasma*, have been reported in livestock in Mongolia [9–14]. The *Anaplasma* species reported in Mongolia include *A. marginale*, *A. phagocytophilum*, and *A. ovis* [13–15]; *A. ovis* infects sheep and goat populations [14,16], of which there were 48.5 million animals in 2015 according to the national statistics census [17]. In addition, *A. ovis* has also been reported in cattle and reindeer in Mongolia [14,18].

Table 1
PCR detection of *A. ovis* in sheep and goats from 19 Mongolian provinces.

Province	Year/month	Sheep			Goat		
		No. sample	No. positive	% (CI[a])	No. sample	No. positive	%(CI)
Arkhangai	2013/7, 2014/7	119	102	85.7 (78.3–90.8)	37	33	89.2 (75.2–95.7)
Bayankhongor	2014/10, 2015/4	50	45	90 (78.6–95.6)	59	47	79.7 (67.7–87.9)
Bayan-Ulgii	2014/8, 2015/4	51	30	58.8 (45.1–71.2)	49	42	85.7 (73.3–92.9)
Bulgan	2013/7, 2014/7	160	136	85 (78.6–89.7)	120	113	94.2 (88.4–97.1)
Dornod	2013/4	18	4	22.2 (9–45.2)	14	5	35.7 (16.3–61.2)
Dornogovi	2014/9	39	6	15.4 (7.2–29.7)	72	17	23.6 (15.3–34.6)
Dundgovi	2013/4, 2014/9	58	28	48.3 (35.9–60.8)	68	23	33.8 (23.7–45.6)
Govi-Altai	2015/4	10	9	90 (59.5–98.2)	16	10	62.5 (38.6–81.5)
Govisumber	2016/6	68	57	83.8 (73.3–90.7)	57	49	86 (74.6–92.7)
Khentii	2013/4, 2015/5	27	2	7.4 (2–23.3)	15	2	13.3 (3.7–37.8)
Khovd	2014/7	49	33	67.3 (53.3–78.7)	50	50	100 (92.9–100)
Khovsgol	2014/7	112	77	68.8 (59.6–76.5)	46	42	91.3 (79.6–96.5)
Omnogovi	2014/9, 2015/4	33	18	54.5 (37.9–70.1)	33	18	54.5 (37.9–70.1)
Ovorkhangai	2014/10	30	28	93.3 (78.6–98.1)	20	18	90 (66.8–98.2)
Selenge	2013/7, 2014/7	110	81	73.6 (64.7–80.9)	68	50	73.5 (61.9–82.5)
Sukhbaatar	2013/4, 2016/5	35	23	65.7 (49.1–79.1)	28	19	67.9 (49.3–82)
Tov	2013/5, 2014/9	109	84	77.1 (68.3–83.9)	40	19	47.5 (32.9–62.5)
Uvs	2016/4	55	23	41.8 (29.7–54.9)	41	31	75.6 (60.6–86.1)
Zavkhan	2014/10	26	14	53.8 (35.4–71.2)	14	12	85.7 (60–95.9)
Total		1179	813	69 (66.2–71.5)	871	621	71.3 (68.2–74.2)

[a] 95% confidence interval.

However, those studies were only conducted in a few Mongolian provinces, and a country-wide survey to determine *A. ovis* infection rates in various Mongolian provinces has not yet been carried out. Additionally, potential tick species associated with *A. ovis* transmission are not known in Mongolia, although identification of tick vectors is very important for devising effective tick control measures to minimise *A. ovis* infection rates. In this study, we surveyed sheep and goats for *A. ovis* infection in 19 of 21 Mongolian provinces using a PCR assay. Additionally, we also screened DNA samples that were extracted from unfed ticks collected in 11 different provinces for *A. ovis* infection.

2. Materials and methods

2.1. DNA samples from sheep, goats, and ticks

Blood samples were collected from 1179 sheep and 871 goats in 19 of 21 Mongolian provinces (Table 1) during 2013–2016. The sampling was not carried out in Darkhan-Uul and Orkhon provinces, formerly known as Darkhan and Erdenet cities, respectively, as the livestock farming is uncommon in these two urban areas. From each animal, approximately 2 ml of whole blood was collected from the jugular vein using a vacutainer tube that contained an anticoagulant (EDTA). All animals were apparently healthy during sampling. All blood samples were subjected to DNA extraction using phenol:chloroform:isoamyl alcohol (25:24:1, *v*/v) [19]. Moreover, a total of 601 questing adult ticks were collected in eight Mongolian provinces (Bayan-Ulgii, Dornod, Govi-Altai, Khovd, Selenge, Omnogovi, Ovorkhangai, and Tov) in 2012 and 2014–2016 using the flagging method. Based on morphology [20,21], the questing ticks were identified as *Dermacentor nuttalli* (*n* = 378), *Ixodes persulcatus* (*n* = 95), and *Hyalomma asiaticum* (*n* = 128). In addition, a total of 120 unfed adult *Haemaphysalis pospelovashtromae*, which was not detected among the questing ticks collected in the present study, were collected from the fur of both sheep and goats in four Mongolian provinces (Arkhangai, Bayankhongor, Bayan-Ulgii, and Zavkhan). The following morphological features were used to identify the tick species; 1) presence of spurs on the leg segments (*D. nuttalli*) or coxae (*Hae. pospelovashtromae*), 2) length of internal spur of coxae (*I. persulcatus* and *D. nuttalli*), 3) shapes of cervical groove, basis capituli, and lateral grooves on scutum (*Hya. asiaticum*), and 4) shape, length, and width of mouth parts (basis capituli, palp, and

hypostome) and shapes of scutum and genital area (*Hae. pospelovashtromae*). Subsequently, individual ticks were digested with a lysis buffer (20 mM Tris–HCl pH 8.0, 1 mM EDTA pH 7.5, 10 mM NaCl, 1% SDS, and 100 µg/ml Proteinase K) as previously described [22], and DNA samples were prepared using phenol:chloroform:isoamyl alcohol (25:24:1, *v*/v) [19]. All DNA samples were stored at −30 °C until further use. All animal procedures were approved by the Committee on the Ethics of Animal Experiments, Obihiro University of Agriculture and Veterinary Medicine (Approval number 28–45).

2.2. PCR detection of A. ovis in sheep, goats, and ticks

All DNA samples from sheep, goats, and ticks were screened for *A. ovis* infection using a previously described major surface protein 4 gene (*msp4*)-based PCR assay [23]. Briefly, a 25-µl reaction mixture was prepared that contained 1.0 µl of template DNA, 1× PCR buffer (10× DreamTaq Buffer, Thermo Fisher Scientific, Vilnius, Lithuania), 200 µM of each dNTPs (Thermo Fisher Scientific), 0.4 µM of each forward (MSP45, 5′-GGGAGCTCCTATGAATTACAGAGAATTGTTTAC-3′) and reverse (MSP43, 5′-CCGGATCCTTAGCTGAACAGGAATCTTGC-3′) primers, 0.25 µl of 5 U/µl *Taq* DNA polymerase (DreamTaq DNA Polymerase, Thermo Fisher Scientific), and 16.75 µl of ultra-pure water. Blood DNA sample from a sheep in Mongolia with *A. ovis* infection confirmed by microscopy as well as by PCR and sequencing [23] was used as a positive control (unpublished data), while a PCR reaction mixture that contained water instead of DNA was used as a negative control. The reaction mixture was then subjected to pre-denaturation at 95 °C for 5 min, and then to 40 cycles of denaturation at 95 °C for 45 s, annealing at 59 °C for 45 s, and extension at 72 °C for 1 min. Final elongation at 72 °C for 5 min was followed by agarose gel electrophoresis, ethidium bromide staining, and visualisation under UV illumination. Detection of a band at approximately 870-bp was considered positive.

2.3. Cloning and sequencing

PCR products with the expected sizes were gel-extracted using NucleoSpin® Gel and PCR Clean-up kit (MACHEREY-NAGGmbH & Co. KG, Düren, Germany). The extracted DNA was ligated to a PCR 2.1
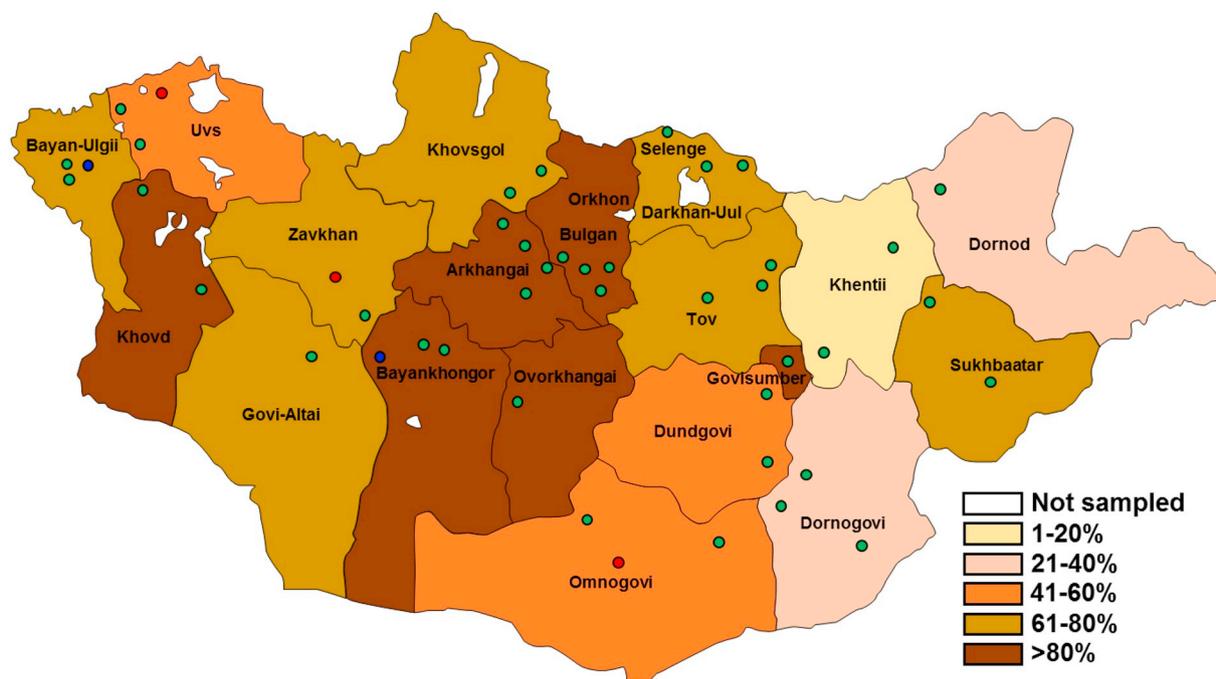
Fig. 1. Epidemiological mapping of *A. ovis* in Mongolia. An epidemiological map was prepared to illustrate the differential *A. ovis* infection rates in small ruminants (both sheep and goats) from 19 Mongolian provinces. The geographical variations of the *A. ovis*-positive rates among small ruminants are indicated by different background colours. The red, blue, and green circles indicate geographical locations at which sheep, goats, and both sheep and goats were sampled, respectively.

plasmid vector (TOPO, Invitrogen, Carlsbad, CA, USA), and the inserts were sequenced using ABI PRISM 3100 genetic analyzer (Applied Biosystems, Branchburg, NJ, USA).

2.4. Sequencing and phylogenetic analyses

The newly generated *msp4* sequences were initially analysed by basic local alignment search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm their origin. The sequences were then trimmed at the both ends to obtain full-length (852-bp) *msp4* sequences. The identity scores shared among the *msp4* sequences were calculated using MatGAT version 2.01 [24]. The newly obtained Mongolian sequences and those obtained from GenBank were used to construct a maximum likelihood phylogeny based on Kimura 2-parameter substitution model [25] using MEGA version 6.0 [26]. The evolutionary rate differences among sites were modelled using a discrete gamma distribution (+G).

2.5. Statistical analyses

The confidence intervals for the *A. ovis*-positive rates were calculated using OpenEpi (http://www.openepi.com/Proportion/Proportion.htm) based on Wilson score interval [27]. The *P* values to assess the statistically significant variations among the rates were calculated using an "N-1" chi-squared test (https://www.medcalc.org/calc/comparison_of_proportions.php) [28,29]. *P* values < .05 were considered to indicate significant variation.

3. Results and discussion

Anaplasmosis caused by *A. ovis* in sheep and goats is widespread in Asian, European, Mediterranean, and North and South American countries [2,30]. This infection may result in clinical disease that leads to severe economic losses [6]. Recent studies detected *A. ovis* in sheep and goats from a few Mongolian provinces [14,31]. However, the country-wide epidemiology of *A. ovis* and tick species that transmit this pathogen were not known in Mongolia. Therefore, the aim of this study was to determine the *A. ovis* infection rates in sheep and goats across

Mongolia, and to identify the potential tick vectors of *A. ovis* in this country.

Anaplasma ovis was detected by the *msp4* PCR assay from both sheep and goats in all surveyed provinces (Table 1). The overall *A. ovis*-positive rates were comparable between sheep and goats analysed in this study. Of 1179 sheep and 871 goat DNA samples, 813 (69.0%) and 621 (71.3%) were positive for *A. ovis* infection. The positive rates were comparable to those determined in several other endemic countries, including Portugal, Sudan, and Iraq [6]. On a per province basis, the positive rates in sheep and goats ranged from 7.4%–93.3% and 13.3%–100%, respectively (Table 1). However, the positive rates were < 40% in small ruminants (sheep and goats) in only three provinces (Dornod, Dornogovi, and Khentii), whereas the positive rates in the rest of the surveyed provinces were > 40% (Fig. 1). In particular, *A. ovis* infection was observed in > 80% of small ruminants from Arkhangai, Bayankhongor, Bulgan, Govisumber, Khovd, and Ovorkhangai (Fig. 1).

In general, animal age is a known risk factor for infections caused by tick-borne pathogens [32–34]. Therefore, we investigated *A. ovis*-positive rates in two different age groups. As the records on the age for 236 sheep and 143 goats were not available, 943 sheep and 728 goats were analysed for *A. ovis* infection in 1–3-year-old and > 3-year-old age groups. However, the positive rates were not different between these age groups in sheep (64.4% and 67.4%, respectively) and goats (80.8% and 72.3%, respectively), which indicates that age is not a risk factor for *A. ovis* infection in Mongolia (Table 2). Although the reason why the positive rates were comparable between age groups is not very clear, the fact that the Mongolian livestock animals, including small ruminants, are extensively managed throughout their life might explain this observation [35]. Therefore, differences in the density and activity of tick vectors that transmit *A. ovis* might be a reason for the differential *A. ovis*-infection rates observed among Mongolian provinces.

Although *A. ovis* has been detected in several tick species, the vectorial capacity of these ticks is unknown, as most of these studies analysed ticks that were collected from the animal body [36–38]. The confirmed tick vectors of *A. ovis* include *Rhipicephalus bursa* and *Dermacentor andersoni* [2], both of which were not reported in Mongolia. In

Table 2
Anaplasma ovis-positive rates in different age groups of sheep and goats from 19 Mongolian provinces.

Province	Sheep							Goat						
	1–3 years			> 3 years				1–3 years			> 3 years			
	No. sample	No. postive	% (CI)[a]	No. sample	No. postive	% (CI)	P value	No. sample	No. postive	% (CI)	No. sample	No. postive	% (CI)	P value
Arkhangai	42	36	85.7 (72.1–93.2)	37	29	78.4 (62.8–88.6)	0.3935	20	19	95.0 (76.3–99.1)	12	11	91.7 (64.6–98.5)	0.7055
Bayankhongor	19	18	94.7 (75.3–99.0)	31	25	80.6 (63.7–90.8)	0.1679	24	19	79.2 (59.5–90.7)	29	24	82.8 (65.4–92.4)	0.7415
Bayan-Ulgii	29	10	34.5 (19.9–52.6)	21	16	76.2 (54.9–89.3)	0.0039	24	22	91.7 (74.1–97.6)	20	19	95.0 (76.3–99.1)	0.6604
Bulgan	86	70	81.4 (71.9–88.2)	63	56	88.9 (78.8–94.5)	0.2133	54	53	98.1 (90.2–99.6)	55	53	96.4 (87.6–99.0)	0.5713
Domod	14	3	21.4 (7.5–47.5)	4	1	25.0 (4.5–69.9)	0.882	10	5	50.0 (23.6–76.3)	4	0	0.0 (0.0–48.9)	
Dornogovi	15	3	20.0 (7.0–45.1)	24	3	12.5 (4.3–31.0)	0.533	4	1	25.0 (4.5–69.9)	42	14	33.3 (21.0–48.4)	0.7378
Dundgovi	22	14	63.6 (42.9–80.2)	23	10	43.5 (25.6–63.1)	0.1794	18	6	33.3 (16.2–56.2)	34	11	32.4 (19.1–49.1)	0.9422
Govi-Altai	3	3	100 (43.8–100)	7	6	85.7 (48.6–97.4)	0.5075	6	4	66.7 (30.0–90.3)	10	6	60.0 (31.2–83.1)	0.7983
Govisumber	31	27	87.1 (71.1–94.8)	37	30	81.1 (65.8–90.5)	0.4318	38	33	86.8 (72.6–94.2)	19	16	84.2 (62.4–94.4)	0.7919
Khentii	20	1	5.0 (0.8–23.6)	7	1	14.3 (2.5–51.3)	0.8002	10	1	10.0 (1.7–40.4)	5	1	20.0 (3.6–62.4)	0.6038
Khovd	25	18	72.0 (52.4–85.7)	24	16	66.7 (46.7–82.0)	0.9531	20	20	100 (83.8–100)	30	30	100 (88.6–100)	
Khovsgol	23	11	47.8 (29.2–67.0)	34	16	47.1 (31.4–63.2)	0.2516	30	26	86.7 (70.3–94.6)	15	15	100 (79.6–100)	
Omnogovi	8	6	75.0 (40.9–92.8)	16	8	50.0 (28.0–72.0)		5	3	60.0 (23.0–88.2)	17	11	64.7 (41.3–82.6)	0.8512
Ovorkhangai	11	11	100 (74.1–100)	19	17	89.5 (68.6–97.0)	0.8905	13	11	84.6 (57.7–95.6)	7	7	100 (64.5–100)	
Selenge	50	36	72.0 (58.3–82.5)	56	41	73.2 (60.4–83.0)	0.0222	33	25	75.8 (58.9–87.1)	35	25	71.4 (54.9–83.6)	0.6902
Sukhbaatar	7	1	14.3 (2.5–51.3)	13	9	69.2 (42.3–87.3)	0.0399	20	18	90.0 (69.9–97.2)	0	0	0	
Tov	13	8	61.5 (35.5–82.2)	28	25	89.3 (72.8–96.2)	0.3173	3	0	0	7	5	71.4 (35.9–91.7)	
Uvs	22	11	50.0 (30.7–69.2)	33	12	36.4 (22.1–53.3)	0.0568	18	16	88.9 (67.2–96.9)	23	15	65.2 (44.8–81.1)	0.0011
Zavkhan	12	4	33.3 (13.8–60.9)	14	10	71.4 (45.3–88.2)		5	5	100 (56.5–100)	9	7	77.8 (45.2–93.6)	0.4215
Total	452	291	64.4 (59.9–68.7)	491	331	67.4 (63.1–71.4)	0.3317	355	287	80.8 (76.4–84.6)	373	270	72.3 (67.6–76.6)	0.5482

[a] 95% confidence interval.

Table 3

PCR detection of *A. ovis* in unfed ticks collected from 11 Mongolian provinces.

Province	Year/month	*Dermacentor nuttalli*[a]		*Ixodes persulcatus*[a]		*Hyalomma asiaticum*[a]		*Haemaphysalis pospelovashtromae*[b]	
		No. samples	No. positive (%)	No. samples	No. positive (%)	No. samples	No. positive (%)	No. samples	No. positive (%)
Arkhangai	2015/4	NC	–	NC	–	NC	–	6	0
Bayankhongor	2015/4, 2016/4	NC	–	NC	–	NC	–	78	0
Bayan-Ulgii	2012/4, 2015/4	136	4 (2.9)	ND	–	ND	–	32	0
Dornod	2016/5	20	0	ND	–	ND	–	NC	–
Govi-Altai	2015/5	48	1 (2.1)	ND	–	ND	–	NC	–
Khovd	2014/5	138	4 (2.9)	ND	–	ND	–	NC	–
Omnogovi	2016/6	ND	–	ND	–	91	0	NC	–
Ovorkhangai	2016/6	ND	–	ND	–	37	0	NC	–
Selenge	2014/6	ND	–	95	0	ND	–	NC	–
Tov	2015/4	36	0	ND	–	ND	–	NC	–
Zavkhan	2015/4	NC	–	NC	–	NC	–	4	0
Total		378	9 (2.4)	95		128		120	0

NC, not collected; ND, not detected.

[a] Questing *Dermacentor nuttalli*, *Ixodes persulcatus*, and *Hyalomma asiaticum* ticks were collected by the flagging method.

[b] Unfed *Haemaphysalis pospelovashtromae* ticks were collected from sheep and goat fur.
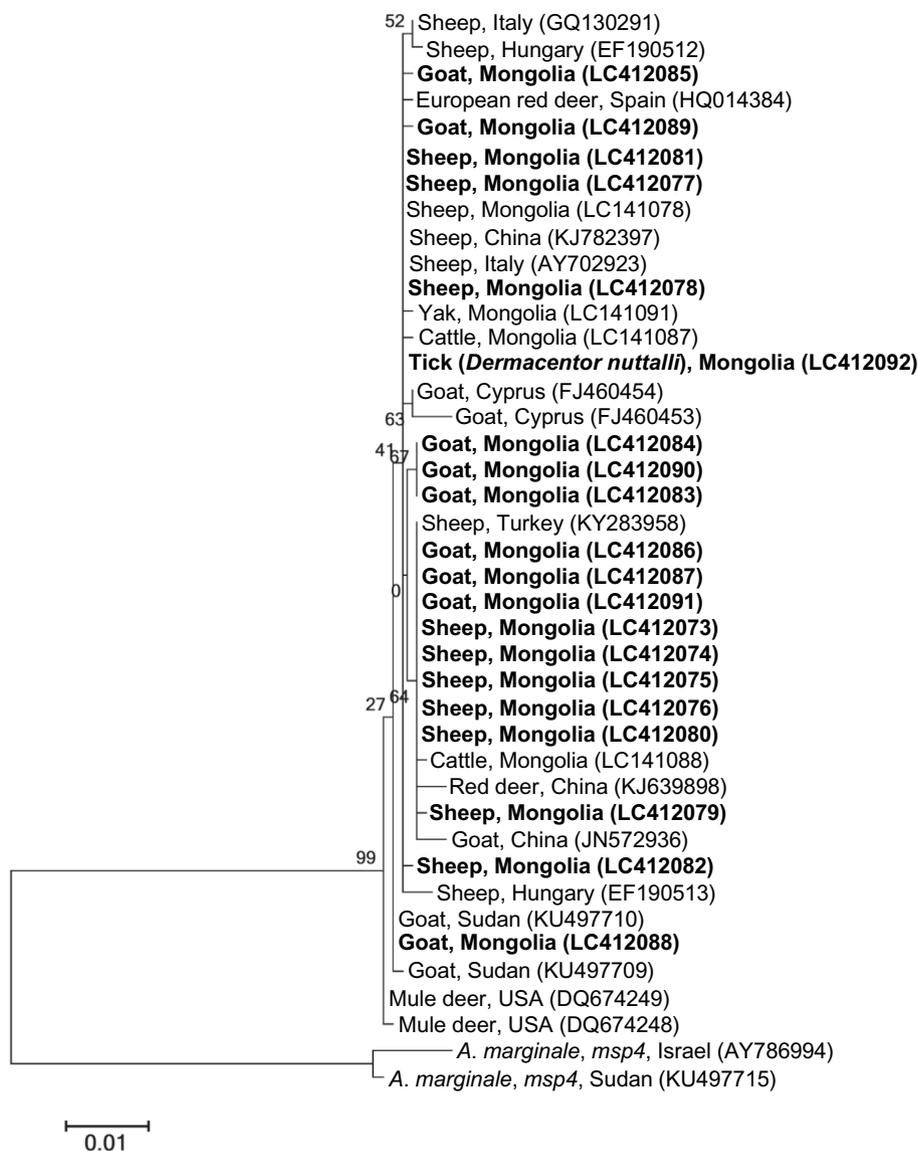

Fig. 2. Phylogenetic tree of *A. ovis msp4*. A maximum-likelihood phylogeny was constructed using the *A. ovis msp4* sequences obtained in this study and those retrieved from GenBank. Two *A. marginale msp4* sequences were used as outgroup sequences. The sequences generated in this study are highlighted in boldface. Note that the newly determined Mongolian gene sequences clustered together with known *A. ovis* sequences from different countries.

addition, a previous study found that *A. ovis* can be transmitted intrastadially (i.e, acquisition and transmission of infection by the same tick in the same stage when moves from one host to other without molting) by *D. nuttalli*, *Hya. asiaticum*, and *Rhipicephalus pumilio*, while transsatadial persistence was not observed [39]. Among these tick species, *D. nuttalli* and *Hya. asiaticum* are endemic in Mongolia, but their involvement in *A. ovis* transmission is unknown. Therefore, to identify potential tick vectors of *A. ovis* in Mongolia, we collected 721 unfed ticks, including 601 questing ticks (*D. nuttalli*, *I. persulcatus*, and *Hya. asiaticum*) collected from pastures in eight provinces and 120 *Hae. pospelovashtromae* that were attached to the fur of sheep and goats in four provinces (Table 3). Among the questing ticks collected, *D. nuttalli* was detected in Bayan-Ulgii, Dornod, Govi-Altai, Khovd, and Tov, whereas *I. persulcatus* was only detected in Selenge. Alternatively, *Hya. asiaticum* was only collected in Omnogovi and Ovorkhangai Provinces (Table 3). The differences in the geography of sampling locations within provinces may explain why only a single tick species was collected in a given province, as the tick distribution in Mongolia varies among steppe, forest, and Gobi areas of each province [40].

When the DNA samples extracted from all 721 ticks were subjected to the *msp4* PCR assay, only nine *D. nuttalli* DNA samples (2.4%) were positive, which indicates that this tick species is a potential *A. ovis* vector in Mongolia. *Anaplasma ovis*-positive *D. nuttalli* ticks were detected in Bayan-Ulgii (2.9%), Govi-Altai (2.1%), and Khovd (2.9%) (Table 3). The positive rates in *D. nuttalli* were, however, very low as compared to those determined in sheep and goats. The present study analysed only unfed adult ticks, and therefore *A. ovis* acquired by the nymphal stage might have been lost when they emerged as adults due to lack of transstadial transmission in *D. nuttalli* [39]. This could explain the low *A. ovis* positive rates in *D. nuttalli* compared with the small ruminants in Mongolia. In this study, we found that *A. ovis* infection rates in sheep and goats were relatively higher in western and central regions compared with the rest of Mongolia, except Sukhbaatar, which is an eastern province (Fig. 1). *Dermacentor nuttalli* abundance in western and central regions might explain the high prevalence of *A. ovis* in these regions, as the high-altitude forest and steppe areas favour colonisation of this tick species [40]. However, the questing ticks collected in Selenge, Omnogovi, and Ovorkhangai did not contain *D. nuttalli*. The differences in the geography of sampling sites within provinces, as discussed elsewhere in this article, may explain why *D. nuttalli* was not detected in these provinces [40].

To confirm the PCR findings, 10, 9, and 1 PCR amplicons with high band intensity from sheep (2 from Khovd, 5 from Govisumber, and 3 from Bayan-Ulgii), goats (3 from Khovd, 4 from Govisumber, and 2 from Bayan-Ulgii), and a *D. nuttalli* tick (from Bayan-Ulgii), respectively, were cloned and sequenced. The resultant *A. ovis msp4* sequences from sheep (GenBank accession numbers: LC412073–LC412082), goats (LC412083–LC412091), and a *D. nuttalli* tick (LC412092) shared 99.6%–100% identity. These sequences also shared 99.5%–100% identity scores with known *A. ovis msp4* sequences from Mongolia (LC141078), China (KJ782397), Italy (AY702923), Turkey (KY283958), Sudan (KU497710), Spain (HQ014384), Cyprus (FJ460454), the USA (DQ674249), and Hungary (EF190512). In the phylogeny, the Mongolian *A. ovis msp4* sequences clustered together with previously reported sequences from Mongolia and with those reported from other endemic countries, which confirmed the PCR findings of this study (Fig. 2).

In conclusion, this study demonstrated that *A. ovis* infects sheep and goats throughout Mongolia, and that *D. nuttalli* is a potential vector in this country. Therefore, *D. nuttalli* control is vital for minimising *A. ovis* prevalence in Mongolia.

Acknowledgements

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