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A PCR-based survey of animal trypanosomes among domestic animals herded together in the Bayan-Ulgii and Khovd provinces of Mongolia

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ABSTRACT
Trypanosoma evansi affects a wide range of domestic animal hosts and can be mechanically cross-transmitted by biting flies when various animals graze together. A total of 1,058 animals (camels, yaks, goats, sheep, cattle, and horses), herded together in the Bayan-Ulgii and Khovd provinces of Mongolia, were screened for animal trypanosomosis using KIN (ITS1) PCR; 21.27% of the samples tested positive. There were significant differences in prevalence among animal species (small ruminants, $p < 0.01$), sex (males, $p < 0.05$), and age (adult, $p < 0.05$). Considering the location, a significant difference in sheep was observed, with the prevalence of trypanosomosis being higher among sheep in Khovd Province ($p < 0.01$) than in Bayan-Ulgii. This is the first study to highlight the prevalence of animal trypanosomosis in domestic animals that range together in Mongolian grasslands. This study also highlights the significance of small ruminants as possible reservoirs of trypanosomes, and shows the relationship between herd structure, age, and sex and trypanosomosis prevalence in Mongolia.

Keywords: Animal trypanosomosis, Domestic animals, Epidemiology, Mongolia

INTRODUCTION
Trypanosoma evansi, belonging to the subgenus *Trypanozoon*, is primarily transmitted mechanically by biting insects because the parasite has lost genetic material and can no longer undergo reproductive cycles in tsetse flies (Lun and Desser, 1995; Lai et al., 2008). *T. evansi* infects several large mammals, causing epidemics of a disease called surra.

Within the same subgenus, *T. equiperdum* causes dourine via sexual transmission in Equidae (Brun et al., 1998).

The livestock sector in Mongolia can generally be regarded as low input and high risk, and the herder's mentality remains geared toward increasing livestock numbers rather than intensification (World Bank, 2009). Previous reports have also shown that the seroprevalence of trypanosomosis in equines in Mongolia is 6% – 8% (Clausen et al., 2003) and 4.1% – 5.5% (Mizushima et al., 2020). However, these studies did not identify the causative species, as it is difficult to distinguish between *T. evansi* and *T. equiperdum* using serological diagnostic techniques. In contrast, *T. equiperdum* was isolated from the urethral tract of a horse showing dourine symptoms in Mongolia, characterized as a true *T. equiperdum* strain, and named the IVM-t1 strain (Suganuma et al., 2016). There are currently no reports of trypanosomosis in other domestic animals in Mongolia. However, local veterinarians have reported clinical symptoms of surra among domestic animals, particularly camels and horses, in the Bayan-Ulgii and Khovd provinces. Therefore, this study aimed to determine the prevalence of animal trypanosomosis among various domestic animals in the Bayan-Ulgii and Khovd provinces of Mongolia. This study provides a baseline for further establishing the prevalence of animal trypanosomosis among various domestic animals herded together under the extensive livestock system in Mongolia, which will help in establishing solutions to mitigate trypanosome infections throughout the country.

MATERIALS AND METHODS

Study area and sample size

Samples were collected from Bayan-Ulgii (48°184.3308″N 89°3046.5768″E) and Khovd provinces (48°22′46.866″N 92°126.9444″E) in northwestern Mongolia during summer (August 2014; Fig. 1). The farming system in these areas is similar to that in the rest of Mongolia, which is nomadic extensive livestock farming characterized by large herds of domestic animals with low input and output. Cattle, sheep, goats, yaks, horses, and camels graze together and move over long distances for pasture. Bayan-Ulgii has a mountainous high-altitude terrain, whereas Khovd is primarily a flat plain. The herd structure of the animals is slightly different in the two provinces and in the Khovd province is characterized by small herds of 100–200 animals scattered on the plains, whereas in Bayan-Ulgii large herds of 500–1,000 animals grazing together are characteristic of this province. Notably, the sheep in Bayan-Ulgii were mainly of Kazakh breed. Blood samples were collected from 1,058 domestic animals (111 camels, 119 yaks, 255 goats, 258 sheep, 100 cattle, and 215 horses) and their sex, age, and sampling province were recorded.

Fig. 1. The map of Mongolia showing the two sampled provinces

Sample collection and DNA preparation

Blood samples were collected from all six domestic animal species, the owners of which consented to participate in the survey. Approximately 5 mL of blood was drawn from the jugular vein of each animal using vacationer tubes containing EDTA-2Na (Terumo Co., Tokyo, Japan). Total DNA from each blood sample was isolated using the phenol-chloroform DNA isolation method (Sambrook et al., 2006). Briefly, 50 μL of blood was suspended in 500 μL of extraction buffer and proteinase K and incubated at 50–55°C for 6 h. The suspension was mixed with an equal volume of phenol-chloroform suspension at pH 8.0, and the resultant water layer containing the DNA was transferred to a new tube. The DNA was then precipitated with an equal volume of isopropanol and a 1/10 volume of 3 mol/L sodium acetate, which was then removed, and the precipitate was air-dried. The resultant DNA pellet was eluted with water and stored at –30°C until further use. This study was approved by the Committee on the Ethics of Animal Experiments of the Obihiro University of Agriculture and Veterinary Medicine (approval number: 28-45).

Detection of trypanosomes using PCR

To detect trypanosomes and identify species, this study used the KIN-PCR technique, which amplified the internal transcribed spacer 1 (ITS1) using primers KIN1 5-GCG TTC AAA GAT TGG GCA AT-3 and KIN2 5-CGC CCG AAA GTT CAC C-3 to obtain a 540 bp product for *Trypanozoon* subspecies amplification (Desquesnes et al., 2001).

Trypanosome DNA was detected using a single-step PCR. The PCR mixtures included 1 μL 10× reaction buffer, 0.3 μL 50 mM magnesium chloride, 1 μL 250 μM dNTPs, and 0.1 μL *Taq* DNA polymerase (all from Thermo Fisher Scientific Inc., MA, USA); 1 μL each of the forward and reverse primers; and 5.1 μL double distilled water with 1 μL DNA sample added to the individual PCR mixtures. PCR was conducted on a thermal cycler (Thermo Fisher Scientific) under the following cycling conditions: initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, annealing at 60°C for 1 min, and

72°C for 1 min, followed by a final extension at 72°C for 7 min, with a resultant amplicon size of 540 bp.

Statistical analysis

Chi-square and Fisher's exact tests were performed, the strengths of their associations were tested, and *p*-values were determined using RStudio Version 1.4.1106. The 95% confidence intervals were calculated using Wilson's score method (Wilson, 1927).

RESULTS

Prevalence of animal trypanosomosis and risk factors

In this study, 225 of the 1,058 sampled animals (21.27%) were positive for animal trypanosomes. The highest prevalence was observed in goats (35.69%), followed by sheep (26.36%), camels (17.12%), horses (15.81%), cattle (7.00%), and yaks (5.04%) (Table 1). The prevalence was significantly different among the host species ($p < 0.01$, Table 1). Moreover, residual analysis revealed that the prevalence in sheep and goats was significantly higher than that in the other species. In addition, significant differences were observed between sexes (males: 25.56%; females: 19.80%; $p < 0.05$), locations (Bayan-Ulgii: 19.82% and Khovd: 37.25%, $p < 0.01$), and age groups (young: 8.96% and adult: 22.10%, $p < 0.05$) (Table 1).

Table. 1. Univariate analysis of trypanosomosis prevalence

Factor		N	P	(%)	[95% CI]	*p*-value
Species	Camel	111	19	17.12	[11.24 - 25.19]	< 0.01
	Yak	119	6	5.04	[2.33 - 10.56]	
	Goat	255	91	35.69*	[30.06 - 41.74]	
	Sheep	258	68	26.36*	[21.36 - 32.05]	
	Cattle	100	7	7.00	[3.43 - 13.75]	
	Horse	215	34	15.81	[11.54 - 21.29]	
Sex	Male	270	69	25.56	[20.72 - 31.08]	< 0.05
	Female	788	156	19.80	[17.16 - 22.72]	
Location	Bayan-Ulgii	454	90	19.82	[16.42 - 23.74]	< 0.01
	Khovd	604	225	37.25	[33.49 - 41.18]	
Age	Young	67	6	8.96	[4.17 - 18.19]	< 0.05
	Adult	991	219	22.10	[19.63 - 24.79]	

N: number of samples, P: number of PCR positive samples

*: The residual analysis showed significant difference ($p < 0.05$) among species

Association of risk factors for animal trypanosomosis prevalence in animal species

There were no significant differences in the prevalence of animal trypanosomosis between the sexes, locations, and age groups in camels, yaks, goats, and cattle (Table 2). In contrast, significant differences were observed in sex (male: 37.50%, female: 22.68%, $p < 0.05$) and location (Bayan-Ulgii: 16.81%, Khovd: 34.53%, $p < 0.01$) of sheep (Table 2). A significant difference was also observed in the location (Bayan-Ulgii: 29.52% and Khovd: 2.73%, $p < 0.01$) of horses (Table 2).

Table. 2. Univariate analysis of trypanosomosis prevalence in each animal species

Species	Factor		N	P	(%)	[95% CI]	*p*-value
Camel	Sex	Male	36	5	13.89	[6.08 - 28.66]	
		Female	75	14	18.67	[11.46 - 28.93]	
	Location	Bayan-Ulgii	5	0	0	[0 - 43.45]	
		Khovd	106	19	17.92	[11.79 - 26.31]	
	Age	Young	10	0	0	[0 - 27.75]	
		Adult	101	19	18.81	[12.39 - 27.52]	
Yak	Sex	Male	3	0	0	[0 - 56.15]	
		Female	116	6	5.17	[2.39 - 10.83]	
	Location	Bayan-Ulgii	93	3	3.23	[1.1 - 9.06]	
		Khovd	26	3	11.54	[4.00 - 28.98]	
	Age	Young	15	0	0	[0 - 20.39]	
		Adult	104	6	5.77	[2.67 - 12.02]	
Goat	Sex	Male	40	17	42.5	[28.51 - 57.81]	
		Female	215	74	34.42	[28.39 - 40.99]	
	Location	Bayan-Ulgii	115	36	31.3	[23.55 - 40.27]	
		Khovd	140	55	39.29	[31.59 - 47.56]	
	Age	Young	0	0	ND	ND	ND
		Adult	255	91	35.69	[30.06 - 41.74]	
Sheep	Sex	Male	64	24	37.50	[26.67 - 49.75]	< 0.05
		Female	194	44	22.68	[17.35 - 29.07]	
	Location	Bayan-Ulgii	119	20	16.81	[11.15 - 24.54]	< 0.01
		Khovd	139	48	34.53	[27.14 - 42.76]	

	Age	Young	0	0	ND	ND	ND
		Adult	258	68	26.36	[21.36 - 32.05]	
Cattle	Sex	Male	8	0	0	[0 - 32.44]	
		Female	92	7	7.61	[3.73 - 14.88]	
	Location	Bayan-Ulgii	17	0	0	[0 - 18.43]	
		Khovd	83	7	8.43	[4.15 - 16.40]	
	Age	Young	3	0	0	[0 - 56.15]	
		Adult	97	7	7.22	[3.54 - 14.15]	
Horse	Sex	Male	119	23	19.33	[13.24 - 27.34]	
		Female	96	11	11.46	[6.52 - 19.36]	
	Location	Bayan-Ulgii	105	31	29.52	[21.65 - 38.85]	< 0.01
		Khovd	110	3	2.73	[0.93 - 7.71]	
	Age	Young	39	6	15.38	[7.25 - 29.73]	
		Adult	176	28	15.91	[11.24 - 22.03]	

N: number of samples, P: number of PCR positive samples, ND: Not determine

DISCUSSION

This is the first study to report the prevalence of animal trypanosomosis among different domestic animal species herded together in Mongolia. In this study, the prevalence of animal trypanosomosis in the two provinces was generally higher (21.27%) than that previously reported (Clausen et al., 2003; Mizushima et al., 2020). This is because the sensitivity of the KIN-PCR used in this study was higher than that of serological or nucleic acid amplification methods used in previous studies and the extensive livestock system in which all the animals are herded together, thus causing cross-infection. However, the shortage of veterinary services and the difficulty of organizing transportation to rural areas to provide these services are also contributing factors (World Bank, 2009). The current study also found that the infection rate among small ruminants (sheep and goats) was significantly higher than that of other domestic animals. This is consistent with the results of our previous study on animal African trypanosomosis in Zambia, which also found a significantly high infection rate among goats (Musinguzi et al., 2016). This is attributed to the disease course in small ruminants being primarily sub-clinical, and therefore, not easily detected by farmers for treatment (Musinguzi et al., 2016) and also due to persisting trypanosome infection in small ruminants (Gutierrez et al., 2006). Additionally, the general value of an individual small ruminant is not comparable to that of the larger animals; therefore, the attention paid to small ruminants by farmers is lower than that for large animals. This implies that small ruminants can easily act as reservoirs of trypanosomosis for the rest of the herd. In addition

to small ruminants, the prevalence in camels (17.12%) and horses (15.81%) was higher than that in bovids (cattle, 7.00%; yaks, 5.04%). A likely reason is that horses and camels having less contact with farmers or herdsmen as they are left to graze by themselves for many days, whereas bovids have daily contact with farmers because of milking; therefore, infections in bovids are easily detected and dealt with. Horses could also have mixed surra and dourine trypanosome infections. Dourine has previously been reported in Mongolia through serosurveillance (Clausen et al., 2003; Mizushima et al., 2020) and clinical cases (Davkharbayar et al., 2020). *T. equiperdum* was also isolated from a horse with dourine symptoms and was characterized as a true *T. equiperdum* strain (Suganuma et al., 2016). However, it is almost impossible to differentiate between *T. evansi* and *T. equiperdum* using PCR methods (Desquesnes et al., 2013).

When the differences between sexes are considered, the prevalence in males was significantly higher than that in females. This is because male animals are not closely managed by farmers, whereas females are in constant contact with farmers, and thus, any abnormal condition or disease can be detected, particularly during milking. In contrast, usually only clinical diseases are detected in males, or they are sometimes found recumbent or dead.

Based on location, the prevalence of trypanosomosis was higher in Khovd than in Bayan-Ulgii. In addition, considering individual animal species, the infection rates among horses and sheep in the two provinces were significantly different. Horses in Bayan-Ulgii had a significantly higher prevalence rate than those in Khovd Province. This can be attributed to the differences in husbandry systems between the two areas: Bayan-Ulgii is primarily a high-altitude hilly area, and livestock are usually kept in large herds belonging to different families. In contrast, Khovd is mostly flat, with livestock kept in small, scattered herds; horses and camels are usually allowed to wander away from other animals. This situation increases the possibility of cross-infection among species in Bayan-Ulgii compared to that in Khovd, particularly among horses. The prevalence of trypanosomosis among sheep in Khovd was significantly higher than that among sheep in Bayan-Ulgii where sheep primarily belong to the Kazakh breed and are adapted to the highland terrain. Elshafie et al. (2013) and Sam-Wobo et al. (2010) revealed differences in trypanosomosis prevalence among horse and cattle breeds, respectively. To the best of our knowledge, there are no previous studies on the differences in trypanosome infection among sheep breeds; however, Kazakh sheep might be less infected than other sheep breeds. This could be because they are more resistant than other sheep breeds.

Regarding age, the prevalence rate was significantly higher in adults than in young animals. Previous studies have also revealed that trypanosomosis prevalence generally increases with age, because elderly animals have a higher risk of infection (Dia et al., 1997; Bhutto et al., 2009; Kyari et al., 2021). In addition, local breeds generally show trypanotolerance without severe symptoms (Naessens et al., 2002; Geert et al., 2009). Almost all samples were collected from Mongolian local breed domestic animals, which may explain the high prevalence observed in adult animals in this study.

This is the first study to document trypanosomosis in multiple animal species herded together in Mongolia and highlights the possibility that small ruminants act as reservoirs for

trypanosomosis in Mongolia. The relationship between the livestock system and the spread of trypanosomosis is also highlighted. We suggest that differences in susceptibility between the Kazakh sheep breed and other sheep breeds are likely. Therefore, this study serves as a baseline for future studies aimed at controlling trypanosomosis in Mongolia. This study was limited by the fact that we could not perform sequence analysis to differentiate between the different *Trypanozoon* species. Therefore, we suggest that in the future, sequence analyses of these samples using different PCR target regions, such as maxicircles and minicircles, should be carried out to differentiate *Trypanozoon* subspecies.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

SUBMISSION DECLARATION AND VERIFICATION

The work described has not been published previously, it is not under consideration for publication elsewhere, and its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out; if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically, without the written consent of the copyright holder.

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