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Original article

Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infections in cattle in Mongolia

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ABSTRACT

Toxoplasma gondii and *Neospora caninum* are protozoan parasites that cause huge economic losses in animal industries worldwide. *N. caninum* can cause abortion storms and high culling rates in cattle, whereas *T. gondii* infection is a significant concern in both human and animals because it can induce abortion and clinical symptoms in immunocompromised hosts. The aim of this study was to determine the seroprevalence of *T. gondii* and *N. caninum* in cattle in Mongolia. Specific antibodies to *T. gondii* and *N. caninum* were detected by using an indirect enzyme-linked immunosorbent assay (iELISA) based on recombinant antigens of dense granule protein 7 of *Toxoplasma gondii* and surface antigen 1 of *Neospora caninum*, respectively. A total of 1438 cattle sera from 20 of 21 provinces of Mongolia and the capital city of Ulaanbaatar were tested. Overall, 18.7% and 26.2% of cattle were positive for specific antibodies to *T. gondii* and *N. caninum*, respectively. Prevalence rates were higher (*T. gondii* infection: $P < .0001$, *N. caninum* infection: $P = .002$) in the central region of Mongolia (*T. gondii* infection: 27.1%, *N. caninum* infection: 30.8%) compared with western region, suggesting that prevalence rates might be influenced by geographical condition, particularly warmer temperatures around this area in Mongolia. The lowest prevalence rates were observed in the western region of Mongolia (*T. gondii*: 9%, *N. caninum*: 20.8%). In addition, the seroprevalence of *N. caninum* in female animals (27.5%) was significantly higher than that in male animals (20.4%) ($P = .018$), suggesting an important risk factor of abortion and stillbirth in cattle. The present results showed that *T. gondii* and *N. caninum* infections might be a risk for public health and economy of the livestock industry in Mongolia. In conclusion, this study demonstrates high seroprevalences of *T. gondii* and *N. caninum* in Mongolia and provides valuable new data for development of control measures against these infections in Mongolia.

1. Introduction

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii,* which infects warm-blooded animals including human and livestock as an intermediate host. Domestic cats and other members of the family *Felidae* serve as definitive hosts of *T. gondii*. At least 17 species of wild felines have been reported as definitive hosts, i.e., European and African wild cats, Pallas' cat, Bobcat, leopard cat, Amur leopard cat, Iriomote cat, Ocelot, Geoffroy's cat, Pampas cat, jaguarundi, cougar, leopard, jaguar, tiger, lion, and cheetah (Tenter et al., 2000). Oocysts shed by the definitive host cause wide-ranging environmental contamination (Hill and Dubey, 2002). *T. gondii* tissue cysts are commonly seen in meat-producing animals such as pig, sheep, and goats, while they are rare in beef and buffalo meat (Tenter et al., 2000). Therefore, raw or undercooked meat from these animals is potentially hazardous if ingested by humans or other animals.

Neosporosis is a serious disease in cattle and dogs caused by the protozoan parasite *Neospora caninum*, which is a *Toxoplasma*-like organism. Major reproductive problems caused by *N. caninum* infection are abortion and stillbirths in cattle (Dubey et al., 2007). Canids, including the Australian dingo (*Canis lupus dingo*) (King et al., 2010), coyote (*Canis latrans*) (Gondim et al., 2004), and gray wolf (*Canis lupus*)

Abbreviations: HRP, horseradish peroxidase; iELISA, indirect enzyme-linked immunosorbent assay; IgG, immunoglobulin G; NcSAG1, surface antigen 1 of *Neospora caninum*; TgGRA7, dense granule protein 7 of *Toxoplasma gondii*.

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Table 1
Seroprevalence of *T. gondii* and *N. caninum* in cattle in different provinces of Mongolia.

Regions of Mongolia	Provinces	Seroprevalence of *T. gondii* % (No. positive / No. sampled)	95% CI of *T. gondii* seroprevalence	Seroprevalence of *N. caninum* % (No. positive / No. sampled)	95% CI of *N. caninum* seroprevalence	Mixed seroprevalence % (No. positive / No. sampled)	95% CI of mixed seroprevalence
Central region	Tov	10.8 (7/65)	4.8–21.5	33.8 (22/65)	22.8–46.7	0.0 (0/65)	0.0–6.9
	Omnogobi	28.7 (29/101)	20.3–38.7	17.8 (18/101)	11.1–26.9	5.9 (6/101)	2.4–12.9
	Gobisumber	20 (4/20)	0.6–44.2	30 (6/20)	12.8–54.3	5.0 (1/20)	0.2–26.9
	Dornogobi	33.3 (14/42)	20.0–49.6	42.9 (18/42)	28.0–58.9	21.4 (9/42)	10.8–37.2
	Selenge	28.2 (20/71)	18.4–40.2	25.4 (18/71)	16.1–37.3	11.3 (8/71)	5.3–21.5
	Dundgobi	42.1 (16/38)	26.7–59.0	44.7 (17/38)	29–61.5	31.6 (12/38)	18.0–48.7
Western region	Gobi-Altai	8.9 (4/45)	2.8–22.1	13.3 (6/45)	5.5–27.4	0.0 (0/45)	0.0–9.0
	Khovd	5.7 (3/53)	1.4–16.6	32.1 (17/53)	20.3–46.4	0.0 (0/53)	0.0–8.0
	Bayan-Olgii	2.6 (4/153)	0.8–6.9	10.5 (16/153)	6.2–16.7	1.3 (2/153)	0.2–5.1
	Uvs	12.3 (8/65)	5.8–23.3	12.3 (8/65)	5.8–23.3	3.1 (2/65)	0.5–11.6
	Zavkhan	20.7 (17/82)	12.8–31.3	43.9 (36/82)	33.1–55.2	12.2 (10/82)	6.3–21.7
Eastern region	Sukhbaatar	17.6 (18/102)	11.0–26.7	17.6 (18/102)	11.0–26.7	3.9 (4/102)	1.2–10.3
	Dornod	17.5 (20/114)	11.2–26.0	34.2 (39/114)	25.7–43.7	4.4 (5/114)	1.6–10.4
	Khentii	15.2 (20/132)	9.7–22.6	18.2 (24/132)	12.2–26.0	4.5 (6/132)	1.8–10.0
Khangai region	Bulgan	23 (14/61)	13.5–35.8	29.5 (18/61)	18.8–42.7	4.9 (3/61)	1.2–14.6
	Khovsgol	20 (11/55)	10.8–33.3	20 (11/55)	10.8–33.3	9.1 (5/55)	3.3–20.7
	Orknon	10 (1/10)	0.5–45.8	20 (2/10)	3.5–55.7	0.0 (0/10)	0–34.4
	Ovorkhangai	41.7 (35/84)	31.1–52.9	38.1 (32/84)	27.9–49.3	21.4 (18/84)	13.5–32
	Bayankhongor	11.8 (9/76)	5.8–21.7	36.8 (28/76)	26.2–48.7	0.0 (0/76)	0.0–6.0
	Arkhangai	17.9 (10/56)	9.3–30.8	25 (14/56)	14.8–38.6	3.6 (2/56)	0.6–13.3
Capital city	Ulaanbaatar	38.5 (5/13)	15.1–64.4	69.2 (9/13)	38.8–89.6	38.5 (5/13)	15.1–67.7
Total		18.7 (269/1438)	16.7–20.8	26.2 (377/1438)	23.9–28.5	6.8 (98/1438)	5.6–8.2

CI: confidence interval.

(Dubey et al., 2011), are definitive hosts and can shed oocysts in their feces. *N. caninum* oocyst-contaminated food or water is considered one route of infection for cattle (De Marez et al., 1999). In addition, vertical transmission of *N. caninum* between dams and calves is another route of the infection (Thurmond et al., 1997; Schares et al., 1998). Although there is no evidence that *N. caninum* infection occurs in humans, anti-*N. caninum* antibodies are detected in humans (Ibrahim et al., 2009; Tranas et al., 1999), suggesting it as a potential zoonotic pathogen.

The agricultural sector is the backbone of the economy in Mongolia. Livestock products, in particular, are considered an important source of income. Mongolian livestock populations (horses, cattle, camels, goats, and sheep) total 66 million head. Among these, the cattle population is estimated to have included 4.3 million individuals in 2017 (National Statistics Office of Mongolia, 2017). Although it is only a subsector of agriculture, livestock produces nearly 30% to the gross domestic product in Mongolia (Shagdar, 2002).

In our previous study, the seroprevalence of *T. gondii* among sheep in seven provinces of Mongolia was examined using an indirect enzyme-linked immunosorbent assay (iELISA) based on recombinant *T. gondii* matrix antigen 1 and latex agglutination test (LAT). The overall prevalence rate of *T. gondii* was 24% (42/175) and 16% (29/175) by iELISA and LAT, respectively (Tumurjav et al., 2010). The seroprevalence of *T. gondii* in the wild Pallas cat (*Otocolobus manul*), a small felid species, was 13% (2/15) (Brown et al., 2005). These results imply the presence of *T. gondii* in Mongolia. In contrast, to our knowledge, there is no report of the prevalence of *N. caninum* in Mongolia. Because the current prevalences of *T. gondii* and *N. caninum* in Mongolia have not been well-studied, epidemiological evidence of the prevalences of *T. gondii* and *N. caninum* in livestock is an urgent issue for reducing not only economic loss of livestock production but also public health risks.

Previous studies have demonstrated that dense granule protein 7 of *T. gondii* (TgGRA7) is a potential diagnostic marker of immunoglobulin G (IgG) in acute and chronic infections. An iELISA based on recombinant TgGRA7 is available to differentiate *T. gondii* infections from other infections with high specificity and sensitivity, in different animals, including goats, sheep, cattle, donkeys, and pigs (Selseleh et al., 2012; Terkawi et al., 2013; Wang et al., 2014; Ichikawa-seki et al., 2015; Fereig et al., 2016). There is substantial agreement between the TgGRA7-based iELISA and LAT (sensitivity and specificity in cattle 84% and 88%, respectively; in sheep 83% and 83%, respectively; in goats 82% and 88%, respectively) (Fereig et al., 2016). Several studies have confirmed that surface antigen 1 (NcSAG1) of *N. caninum* is a useful diagnostic antigen that can be used to detect specific antibodies against *N. caninum* in cattle during acute and chronic infections (Chahan et al., 2003; Wilkovsky et al., 2011; Hiasa et al., 2012; Takashima et al., 2013; Ichikawa-seki et al., 2016). Thus, the aim of the present study was to conduct a large-scale examination of the seroprevalence of *T. gondii* and *N. caninum* infections and risk factors for such infection in cattle in Mongolia using iELISAs based on the recombinant proteins TgGRA7 and NcSAG1.

2. Materials and methods

2.1. Ethics statement

This study was performed in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the Ministry of Education, Culture, Sports, Science and Technology, Japan. The protocol was approved by the Committee on the Ethics of Animal Experiments at Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan (permit number 18–15).

2.2. Study area and samples

Based on economic and geographical conditions, Mongolia is divided into four major regions comprising 21 provinces: central region (Tov, Omnogobi, Gobisumber, Dornogobi, Selenge, and Dundgobi), western region (Gobi-Altai, Khovd, Bayan-Olgii, Uvs, and Zavkhan), eastern region (Sukhbaatar, Dornod, and Khentii) and khangai region (Khovsgol, Arkhangai, Ovorkhangai, Bayankhongor, Bulgan, and Orknon). A total of 1438 cattle sera from 20 provinces of Mongolia and the capital city of Ulaanbaatar were tested in this study (Table 1, Figs. 1 and 2); no samples were available from Darkhan-Uul province, which is located in the central region of Mongolia. Cattle sera collected from 2014 to 2016 were obtained from the collection of serum samples at the