Title

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

Running title

Distribution of *Toxoplasma* and *Neospora* in cattle in Mongolia

Authors

Baldorj Pagmadulam[a], Punsantsogvoo Myagmarsuren[b], Ragab M. Fereig[a,c], Makoto Igarashi[a], Naoaki Yokoyama[a], Badgar Battsetseg[b], Yoshifumi Nishikawa[a,*]

Addresses

[a]*National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan*

[b]*Institute of Veterinary Medicine, Laboratory of Molecular Genetics, Mongolian University of Life Sciences, Zaisan 17024, Ulaanbaatar, Mongolia*

[c]*Department of Animal Medicine, Faculty of Veterinary Medicine, South Valley University, Qena City, Qena 83523, Egypt*

[*]Corresponding author at: Obihiro University of Agriculture and Veterinary Medicine, Inada-Cho, Obihiro, Hokkaido 080-8555, Japan; Tel: +81-155-49-5886; Fax: +81-155-49-5643; E-mail: nisikawa@obihiro.ac.jp (Y. Nishikawa)

Authors' contributions

Conceived and designed the experiments: BP, BB, NY, YN.

Performed the experiments: BP, RF, YN.

Analyzed the data: BP, PM, YN.

Contributed reagents/materials/analytical tools: BP, PM, RF, MI, YN.

Wrote the manuscript: BP, YN.

Critically revised the manuscript: BP, BB, NY, YN.

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*.

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 1,438 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: $P < 0.0001$, *N. caninum* infection: $P = 0.002$) 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%) ($P = 0.018$), 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.

Keywords: *Toxoplasma gondii*; *Neospora caninum*; Seroprevalence; Cattle; 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 et al., 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*) (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

85 (horses, cattle, camels, goats, and sheep) total 66 million head. Among these, the cattle
86 population is estimated to have included 4.3 million individuals in 2017 (National Statistics
87 Office of Mongolia, 2017). Although it is only a subsector of agriculture, livestock produces
88 nearly 30% to the gross domestic product in Mongolia (Shagdar, 2002).

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

100 Previous studies have demonstrated that dense granule protein 7 of *T. gondii* (TgGRA7)
101 is a potential diagnostic marker of immunoglobulin G (IgG) in acute and chronic infections.
102 An iELISA based on recombinant TgGRA7 is available to differentiate *T. gondii* infections
103 from other infections with high specificity and sensitivity, in different animals, including goats,
104 sheep, cattle, donkeys, and pigs (Selseleh et al., 2012; Terwaki et al., 2013; Wang et al., 2014;
105 Ichikawa-seki et al., 2015; Fereig et al., 2016). There is substantial agreement between the
106 TgGRA7-based iELISA and LAT (sensitivity and specificity in cattle 84% and 88%,
107 respectively; in sheep 83% and 83%, respectively; in goats 82% and 88%, respectively) (Fereig
108 et al., 2016). Several studies have confirmed that surface antigen 1 (NcSAG1) of *N. caninum*
109 is a useful diagnostic antigen that can be used to detect specific antibodies against *N. caninum*

in cattle during acute and chronic infections (Chanan et al., 2003; Wilkowsky 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 1,438 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 Institute of Veterinary Medicine in Mongolia. Blood samples were collected with venal puncture into glass tubes without anticoagulant from each animal in

the field. The blood samples were placed in an icebox and immediately transferred to the laboratory for the preparation of sera. They were kept frozen at −30°C until analysis. Samples were divided according to region, province, age group (1–4, 5–9, and 10–14 years), and sex for risk factor analyses of the infections.

2.3. Serological testing

In the present study, recombinant proteins of NcSAG1 and TgGRA7 were used for detection of specific antibodies (Terwaki et al., 2013; Chahan et al., 2003). Purified NcSAG1 fused with glutathione S-transferase (GST) was used for detection of *N. caninum*-specific antibodies by iELISA. iELISA based on recombinant TgGRA7 protein was used for detection of anti-*T. gondii* antibodies. Fifty microliters of purified antigen were coated onto ELISA plates (Nunc, Roskilde, Denmark) with a coating buffer (carbonate-bicarbonate buffer, Sigma, St. Louis, MO, USA) at a final concentration of 0.1 µM and incubated at 4°C overnight. After incubation, plates were washed with washing buffer [0.5% Tween 20 in phosphate-buffered saline (PBS)], blocked with 100 µl of blocking solution (3% skim milk in PBS) in each well, and incubated at 37°C for 1 h. Plates were washed with washing buffer once. Cattle sera were diluted at 1:200 with 3% skim milk in PBS, and then 50 µl of positive control, negative control, or test serum sample were added to the wells and incubated at 37°C for 1 h. After washing the plates six times with washing buffer, 50 µl of horseradish peroxidase (HRP)-conjugated anti-bovine IgG (Bethyl Laboratories, Montgomery, TX, USA) diluted at 1:10,000 (1:1 v/v in glycerol) with 3% skim milk in PBS were added to each well and incubated at 37°C for 1 h. After washing the plates six times with washing buffer, 50 µl of TMB substrate reagent (BD Bioscience, San Diego, CA, USA) were quickly added to each well and incubated at room temperature for 10 min in the dark. After incubation, 50 µl of stop solution (1 M H_2SO_4) were added to each well. Absorbance values at 450 nm (A_{450}) of each reaction were determined

using an ELISA reader. The readings for recombinant antigens were subtracted from those of GST protein. The cut-off point was determined as the mean A_{450} value for standard negative sera kept in our laboratory (n = 8 negative sera) plus 3 standard deviations. The standard positive and negative sera used for the *Neospora* study were confirmed with a commercial indirect fluorescent antibody test (IFAT; VMRD Inc., Pullman, WA, USA). The sera from experimentally infected cattle were used as the standard positive sera (Nishimura et al., 2013). The standard negative sera used for the *Toxoplasma* study were confirmed with a commercial LAT (Toxocheck-MT, Eiken Chemical, Tokyo, Japan). Although we had no sera from experimentally *T. gondii*-infected cattle, the reactivity of the recombinant TgGRA7 to field bovine sera was confirmed in our previous studies (Ichikawa-Seki et al., 2015; Fereig et al., 2016). In those studies, the specificity and sensitivity between a commercial LAT (Toxocheck-MT) and the TgGRA7-based iELISA for cattle sera were calculated (sensitivity 84%, specificity 88%; Fereig et al., 2016). In the case, the specificity and sensitivity between an IFAT and an NcSAG1-based iELISA for cattle sera were also calculated (sensitivity 100%, specificity 100%; Chahan et al., 2003).

2.4. Statistical analysis

The data analysis was performed with the GraphPad Prism 6 software (GraphPad software Inc., La Jolla, CA, USA). A χ^2 test was used to analyze the data. Associations were tested with odds ratios (ORs) and 95% confidence intervals after adjustment. A P value of < 0.05, determined with the VassarStats online tool (http://vassarstats.net/), was considered to indicate statistically significant differences.

3. Results

The overall seroprevalence rate of *T. gondii* in Mongolian cattle was 18.7% (range: 2.6–

42.1%) (Table 1). The highest seroprevalence rate of *T. gondii* was 42.1% in Dundgobi, which is located in the central region of Mongolia. In addition, higher seroprevalence rates were observed in Ovorkhangai of khangai region (41.7%) and the capital city of Ulaanbaatar (38.5%). Lower seroprevalence rates were seen in Bayan-Olgii (2.6%) and Khovd (5.7%) provinces in the western region (Table 1, Fig. 1). Higher seroprevalence rates of *T. gondii* were found in the central region (27.1%) and the khangai region (23.3%) compared with that of the western region (9.0%), which had the lowest seroprevalence rate (Table 2). This result suggests that geographical condition may be a potential risk factor of *T. gondii* infection in Mongolia. Although age and sex were also analyzed as potential risk factors of *T. gondii* infection, no significant differences were observed (Table 3).

The overall seroprevalence of *N. caninum* in cattle of Mongolia was 26.2% (range: 10.5%–69.2%) (Table 1). The highest seroprevalence rate was observed in the capital city of Ulaanbaatar (69.2%). In addition, Dornogobi (42.9%) and Dundgobi (44.7%) provinces in the central region and Zavkhan province (43.9%) in the western region showed higher seroprevalence rates. The three lowest seroprevalence rates were seen in the western region, Bayan-Olgii (10.5%), Uvs (12.3%) and Gobi-Altai (13.3%) (Table 1, Fig. 2). The seroprevalence rates of *N. caninum* in the central region (30.8%) and the khangai region (30.7%) were higher than that in the western region (20.8%) (Table 4). Risk factor analysis of *N. caninum* seroprevalence showed no significant difference according to age, while a significant difference in *Neospora* seroprevalence was observed according to sex (Table 5). In particular, the seropositive rate of female animals (27.5%) was higher than that of male animals (20.4%) ($P = 0.018$).

Mixed infections of *T. gondii* and *N. caninum* were also observed among the cattle in Mongolia. The overall seroprevalence of mixed infections was 6.8% and the infection rates in different provinces ranged from 0% to 38.5% (Table 1). Higher rates of mixed infections were

observed in Ulaanbaatar (38.5%), Dundgobi (31.6%), Dornogobi (21.4%), and Ovorkhangai (21.4%) than in the other provinces, in which they ranged from 0% to 12.2% (Table 1).

4. Discussion

The livestock sector plays an important economic role in Mongolia because milk and meat products derived from cattle are served as foods in the daily life of Mongolians. The meat most frequently consumed in Mongolia is from sheep (31.5%), goats (27.7%), and cattle (21.3%). An annual report of the milk industry in Mongolia in 2016 estimated the milk production from cows, sheep, and goat to be 522.4, 99.1, and 173.6 million liters, respectively. Therefore, cattle are one of the most important food sources in Mongolia (National Statistics Office of Mongolia, 2017). Although *T. gondii* or *N. caninum* infection affects the livestock industry by decreasing productivity due to abortion and stillbirth in cattle and neurological symptoms in calves, available data of the prevalences of these protozoan parasites in Mongolia are limited. Therefore, in the present study, cattle sera from 20 of 21 provinces of Mongolia were examined for seropositivity to *T. gondii* and *N. caninum*. Overall seroprevalences of *T. gondii* and *N. caninum* in cattle of Mongolia were 18.7% and 26.2%, respectively. The mixed infection rate was 6.8%. China and Russia are neighboring countries of Mongolia. In China, the prevalence rate of *T. gondii* in cattle was 5.7% in the southern region when determined with an indirect hemagglutination antibody test (Zhou et al., 2012) and 4.8% in the northwest region when determined with a modified agglutination test (Tan et al., 2015). However, the prevalence of *Toxoplasma* in cattle in Russia has not been reported. When the seroprevalence of *N. caninum* in cattle was determined with an ELISA, the prevalence rates were 13.3% in northeastern China (Wang et al., 2010), 18.9% in southern China (Xia et al., 2011), and 9.97% in Russia (Hemphill and Gottstein, 2000). Thus, the seroprevalences of *T. gondii* and *N. caninum* in cattle of

234 Mongolia were higher than those reported in China and Russia, indicating that *T. gondii* and
235 *N. caninum* infections may be a hazard to the livestock industry in Mongolia.

236 In our study, both *N. caninum* and *T. gondii* prevalence rates were significantly higher
237 in the central region of Mongolia, suggesting that one risk factor of such infections might be
238 climate condition, particularly warmer temperatures around this area. In contrast, the overall
239 lowest *T. gondii* prevalence rate was observed in the western region of Mongolia. According
240 to climate and geographical conditions, the average annual temperature of the central region is
241 higher because most of the largest desert (Gobi) is located around this area. Conversely, the
242 western region of Mongolia is the coldest and has the highest elevation (Altai Mountain area).
243 Although *T. gondii* oocysts (and likely *N. caninum* oocysts) are resistant to environmental
244 conditions such as high temperature, drying, and freezing (Dubey et al, 1970; Dubey et al,
245 1998), these infections may increase more readily in warmer and humid conditions than in
246 colder and drier climates (Tenter et al., 2000). Thus, climate and geographical conditions in
247 Mongolia may affect both *N. caninum* and *T. gondii* seroprevalence rates.

248 *Toxoplasma gondii* is not the main causative agent of abortion in cattle, and the
249 contribution of milk and meat from infected cattle to the prevalence of *Toxoplasma* in humans
250 is unknown (Dubey, 1986). However, the consumption of undercooked or raw beef and cow's
251 milk are considered risk factors for human infection. In Mongolia, although the prevalence of
252 *T. gondii* in cattle may be an infectious source to human, human *T. gondii* infection is not well
253 studied. The TORCH test is a blood screen for *T. gondii*, rubella virus, cytomegalovirus, herpes
254 simplex, human immunodeficiency virus, and other organisms, which lead to severe fetal
255 anomalies and fetal loss (Kaur et al 1999). The TORCH test is recommended for pregnant
256 women with high-risk pregnancies in Mongolia. Although TORCH infections were tested
257 among 100 pregnant women in Mongolia, *T. gondii* infection was not detected by ELISA
258 (Otgontsetseg et al., 2013). However, large-scale screening of *T. gondii* is required to

understand the impact of toxoplasmosis in Mongolia. Meat and meat-derived products containing *T. gondii* tissue cysts are potential sources of infection in human (Tenter et al., 2009). However, consumption of undercooked and raw meat is not a typical practice among Mongolians. Therefore, intake or consumption of contaminated water, soil, and raw vegetables might be a potential risk factor of *T. gondii* infection in human.

Although another source of *T. gondii* infection is cats and members of the family *Felidae*, cats are not common domestic animals in Mongolia. Generally, domestic cats are not widespread in any region of Mongolia because herders do not usually keep cats as pets. However, small numbers of domestic cats are kept in households to control rodents. Several species of wild cats inhabit Mongolia such as the wild cat (*Felis silvestris*), Eurasian lynx (*Lynx lynx*), Pallas's cat (*O. manul*), and snow leopard (*Uncia uncia*). Among them, wild cats are distributed in desert areas (Gobi) and Mongolian Altai Mountain area. However, hybridization between wild and domestic cats has been observed, which influences the genetic purity of wild cats, and competition between prey domestic cats and wild cats can lead to disease transmission. The Eurasian lynx (*L. lynx*) is widely distributed in Mongolia (Clark et al., 2006). The snow leopard is distributed in the mountainous area of western Mongolia, but they are rare in all parts of Mongolia. The snow leopard is also thought to prey on livestock (Mallon 1984; Clark et al., 2006; Shehzad et al., 2012). Thus, the transmission of *T. gondii* infection via oocysts shed by wild felines may also facilitate infection in human (especially nomads) and livestock.

In the present study, the seroprevalence of *N. caninum* was significantly higher in female cattle (27.5%) compared with male cattle (20.4%), suggesting sex is an important risk factor because of expected reproductive problems such as abortion and stillbirth in cattle. *N. caninum*-specific antibody titers increase during pregnancy and peak within a month after calving in *N. caninum*-infected dams (Ybañez et al., 2013). Therefore, the detection rate of *N. caninum*-

specific antibodies in female cattle might be higher than that in male animals. However, this result may be attributable to the unequal sample sizes tested (female: 1,174, male: 264).

Furthermore, the *N. caninum* infection rate in cattle was shown to increase in the presence of dogs on farms as a neighborhood animal (Dubey et al., 2007). Mongolian herders keep dogs in their households as herding dogs. Although most herders and farmers maintain several dogs, they generally do not check the health of their dogs or medicate them. Therefore, the high prevalence of *N. caninum* infection in Mongolian cattle may be attributable to their contact with the feces of *N. caninum*-infected domestic dogs. Consequently, further studies are urgently required to determine the infection rate of *N. caninum* in dogs in this region to better understand the infection source. In addition, wild canids should be considered as the definitive host of *N. caninum*. Wild canids such as gray wolf (*Canis lupus*), Asiatic wild dog (*Cuon alpinus*), raccoon dog (*Nyctereutes procyonoides*), and corsac fox (*Vulpes corsac*) are distributed in Mongolia. Among them, the gray wolf is widely distributed and considered prey livestock in Mongolia (Clark et al., 2006). However, to our knowledge, there are no reports about the prevalence of *N. caninum* infection in these dogs in Mongolia. Further investigations are needed to assess the relationship between dogs and cattle for *N. caninum* infection in Mongolia.

5. Conclusions

We conducted the first study of the seroprevalence of *T. gondii* and *N. caninum* infections among cattle in Mongolia. Because the seroprevalences of the infections in cattle of Mongolia were higher than those reported in China and Russia, *T. gondii* and *N. caninum* infections may be a hazard to the livestock industry in Mongolia. Additionally, the seroprevalence rates were affected by climate and geographical conditions in Mongolia. However, to understand the distribution patterns of such infections, identify major risk factor(s), and establish more potent

309 control measures, additional surveys for toxoplasmosis and neosporosis in Mongolia among
310 different livestock are required.
311
312 **Conflict of interests**
313 All authors declare that they have no financial or personal conflicts.
314
315 **Acknowledgements**
316 This study was supported by research grants from the Ito Foundation of Japan (154), the
317 AMED/JICA, and the Science and Technology Research Partnership for Sustainable
318 Development (SATREPS). We would like to thank all members of the Laboratory of Molecular
319 Genetics at the Institute of Veterinary Medicine, Mongolian University of Life Sciences in
320 Mongolia, for their valuable help. We also thank Christina Croney, PhD, from Edanz Group
321 (www.edanzediting.com/ac), for editing a draft of this manuscript.
322

323 **References**

324 Brown, M., Lappin, M. R., Brown L. J., Munkhtsog, B., Swanson, F.W., 2005. Exploring the
325 ecological basis for extreme susceptibility of Pallas' cats (*Octolobus Manul*) to fatal
326 Toxoplasmosis. J. Wildl. Dis. 41, 691-700.

327 Chahan, B., Gaturaga, I., Huang, X., Liao, M., Fukumoto, S., Hirata, H., Nishikawa, Y.,
328 Suzuki, H., Sugimoto, C., Nagasawa, H., Fujisaki, K., Igarashi, I., Mikami, T., Xuan, X.,
329 2003. Serodiagnosis of *Neospora caninum* infection in cattle by enzyme-linked
330 immunosorbent assay with recombinant truncated NcSAG1. Vet. Parasitol. 118, 177-185.

331 Clark, E. L., Munkhbat, J., Dulamtseren, S., Baillie, J. E. M., Batsaikhan, N., Samiya, R. and
332 Stubbe, M. (compilers and editors), 2006. Mongolian Red List of Mammals. Regional
333 Red List Series Vol. 1. Zoological Society of London, London. 94-100.

334 De Marez, T., Liddell, S., Dubey, J.P., Jenkins, M.C., Gasbarre, L., 1999. Oral infection
335 of calves with *Neospora caninum* oocysts from dogs: humoral and cellular immune
336 responses. Int. J. Parasitol. 29, 1647-1657.

337 Dubey, J.P., Miller, N.L., Frenkel, J.K., 1970. The *Toxoplasma gondii* oocyst from cat feces.
338 J. Exp. Med. 132, 636-662.

339 Dubey, J.P., 1986. A review of Toxoplasmosis in cattle. Vet. Parasitol. 22, 177-202.

340 Dubey, J.P., 1998. *Toxoplasma gondii* oocyst survival under defined temperatures. J. Parasitol.
341 84 (4), 862-865.

342 Dubey, J.P., Schares, G., Ortega-Mora, L.M., 2007. Epidemiology and Control of Neosporosis
343 and *Neospora caninum*. Clin. Microbiol. Rev. 20, 323-367.

344 Dubey, J.P., Jenkins, M.C., Rejendran, C., Miska, K., Ferreira, L.R., Martins, J., Kwok,
345 O.C.H., Choudhaey, S., 2011. Gray wolf (*Canis lupus*) is a natural definitive host for
346 *Neospora caninum*. Vet. Parasitol. 181, 382-387.

347

348 Fereig, R.M., Mahmoud, H.Y.A.H., Mohamed S.G.A., AbouLaila, M.R., Abdel-Wahab, A.,
349 Osman, S.A., Zidan, S.A., El-Khodary, S.A., Mohamed, A.E.A., Nishikawa, Y., 2016.
350 Seroprevalence and epidemiology of *Toxoplasma gondii* in farm animals in different
351 regions of Egypt. Vet. Parasitol: Reg. Stud. Rep. 3–4, 1–6.
352 Gondim, L.F.P., McAllister M.M., Pitt, W.C., Zemlicka, D. E., 2004. Coyotes (*Canis latrans*)
353 are definitive hosts of *Neospora caninum*. Int. J. Parasitol. 34, 159-161.
354 Hemphill, A., Gottstein, B., 2000. A European perspective on *Neospora caninum*. Int. J.
355 Parasitol. 30, 877-924.
356 Hiasa, J., Kohara, J., Nishimura, M., Xuan, X., Tokimitsu, H., Nishikawa, Y. 2012. ELISAs
357 based on rNcGRA7 and rNcSAG1 antigens as an indicator of *Neospora caninum*
358 activation. Vet. Parasitol. .187, 379-385.
359 Hill, D., Dubey, J.P., 2002. *Toxoplasma gondii:* transmission, diagnosis and prevention. Clin.
360 Microbial. Infect. 8, 634-640.
361 Ibrahim, H.M., Huang, P., Salem, T.A., Talaat, R.M., Nasr, M.I., Xuan, X., Nishikawa, Y.,
362 2009. Short report: prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in
363 northern Egypt. Am. J. Trop. Med. Hyg. 80, 263-267.
364 Ichikawa-Seki, M., Guswanto, A., Allamanda, P., Mariamah, S.E., Wibowo, E.P., Ihaeashi, I.,
365 Nishikawa, Y., 2015. Seroprevalence of antibody to TgGRA7 antigen of *Toxoplasma*
366 *gondii* in livestock animals from Western Java, Indonesia. Parasitol Int. 64, 484-486.
367 Ichikawa-Seki, M., Guswanto, A., Allamanda, P., Mariamah, S.E., Wibowo, E.P., Nishikawa,
368 Y., 2016. Seroprevalence of antibody to NcSAG1 antigen of *Neospora caninum* in cattle
369 from western Java, Indonesia. J Vet Med Sci. 78, 121-123
370 Kaur, R., Gupta, N., Nair, D., Kakkar, M., Mathur M.D., 1999. Screening for TORCH
371 infections in pregnant women: A report from Delhi. Southeast Asian J. Trop. Med. Pub.
372 Health. 30, 284-286.

King, J.S., ŠIapeta, J., Jenking, D.J., Al-Qassab, S.E., Ellis, J.T., Windsor P.A., 2010. Australian dingoes are definitive hosts of *Neospora caninum*. Int. J. Parasitol. 40, 945-950.

Mallon, D., 1984. The snow leopard, *Panthera uncia,* in Mongolia. 98, Wilbraham road, Manchester M14 7DR, Great Britain.

National Statistics Office of Mongolia, 2017. http://mofa.gov.mn/exp/blog/7/3#, Accessed date: 4 July 2018

Nishimura, M., Hohara, J., Kuroda, Y., Hiasa, J., Tanaka, S., Muroi, Y., Kojima, N., Furuoka, H., Nishikawa, Y., 2013. Oligomannose-coated lissome-entrapped dense granule protein 7 induces protective immune response to *Neospora caninum* in cattle. Vaccine. 31, 3528-3535.

Otgontsetseg, E., Erdenebayar, N., Sarantuya, J., 2013. Detection of TORCH infection among pregnant women. (Жирэмсэн эмэгтэйчүүдийн дунд *Toxoplasma gondii*-ийн халдварыг илрүүлсэн дүн). http://mongolmed.mn/article/4410, Accessed date: 4 October 2017.

Selseleh, M., Keshavarz, H., Mohebali, M., Shojaee, S., Selseleh, M., Eshragian, R.M., Mansouri, F., Modarressi, H.M., 2012. Production and Evaluation of *Toxoplasma gondii* recombinant GRA7 for serodiagnosis of Human infections. Korean J Parasitol. 50, 233-238.

Schares, G., Peters, M., Wurm, R., Bärwald, A., Conraths, F.J., 1998. The efficiency of vertical transmission of *Neospora caninum* in dairy cattle analyzed by serological techniques. Vet. Parasitol. 80, 87-98.

Shagdar, E., 2002. The Mongolian livestock sector: vital for the economy and people, but vulnerable to natural phenomena. Erina Rep. 47. 4-26.

Shehzad, W., McCarthy, T.M., Pompanon, F., Purevjav, L., Coissac, E., Riaz, T., Taberlet, P, 2012. Prey preference of Snow Leopard (*Panthera uncia*) in South Gobi, Mongolia. PloS One. 7, e32104.

Takashima, Y., Takasu, M., Yanagimoto, I., Hattori, N., Batanova, T., Nishikawa, Y., Kitoh, K., 2013. Prevalence and dynamics of antibodies against NcSAG1 and NcGRA7 antigens of *Neospora caninum* in cattle during the gestation period. J. Vet. Med. Sci. 75, 1413-1418.

Tan, Q.D., Yang, X.Y., Yin., M.Y., Hu, L.Y., Qin, S.Y., Wang J.L., Zhou, D.H., Zhu, X.Q. 2015. Seroprevalence and correlates of *Toxoplasma gondii* infection in dairy cattle in northwest China. Acta. Parasitol. 60, 618-621.

Terkawi, M.A., Kameyama, K., Rasul, N.H., Xuan, X., Nishikawa, Y., 2013. Development of an Immunochromatographic Assay Based on Dense Granule Protein 7 for Serological Detection of *Toxoplasma gondii* Infection. Clin. Vaccine Immunol. 20, 596–601.

Tenter, A.M., Heckeroth, A.R. and Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. 30, 1217–1258.

Tenter, A.M., 2009. *Toxoplasma gondii* in animals used for human consumption. Mem. Inst. Oswaldo Cruz. 104, 364-369.

Tranas, J., Heinzen, R.A., Weiss, L.M., McAllister, M.M., 1999. Serological evidence of human infection with the protozoan *Neospora caninum*. Clin. Diagn. Lab. Immunol. 6, 765-767.

Tumurjav, B., Terkawi, M.A., Zhang, H., Zhang, G., Jia, H., Goo, Y.K., Yamagishi, J., Nishikawa, Y., Igarashi, I., Sugimoto, C., Xuan, X., 2010. Serodiagnosis of ovine toxoplasmosis in Mongolia by an enzyme-linked immunosorbent assay with recombinant *Toxoplasma gondii* matrix antigen 1. Japan. J. Vet. Res. 58, 111-119.

420 Thurmond, M.C., Hietala, S.K., Blanchard, P.C., 1997. Herd-based diagnosis of *Neospora*
421 *caninum*-induced endemic and epidemic abortion in cows and evidence for congenital
422 and postnatal transmission. J. Vet. Diagn. Invest. 9, 44-49.
423 Wang, C., Wang, Y., Zou, X., Zhai, Y., Gao, J., Hou, M., Zhu, X.Q., 2010. Seroprevalence
424 of *Neospora caninum* Infection in Dairy Cattle in Northeastern China. J. Parasitol. 96,
425 451-452.
426 Wang, Z., Ge, W., Li, J., Song, M., Sun, H., Wei, F., Liu, Q., 2014. Production and evaluation
427 of recombinant granule antigen protein GRA7 for serodiagnosis of *Toxoplasma gondii*
428 infection in cattle. Foodborne pathogens and disease. 11, 734-739.
429 Wilkovsky, E.S., Bareiro, G.G., Mon, L.M., Moore, P.D., Caspe, G., Campero, C., Fort, M.,
430 Romano, I.M., 2011. An applied printing immunoassay with recombinant Nc-SAG1 for
431 detection of antibodies to *N. caninum* in cattle. Journal of Veterinary diagnostic
432 Investigation. 23, 971-976.
433 Xia, H.Y., Zhou, D.H., Jia, K., Zeng, X.B., Zhang, D.W., She, L.X., Lin, R.Q., Yuan, Z.G., Li,
434 S.J., Zhu, X.Q., 2011. Seroprevalence of *Neospora caninum* Infection in Dairy Cattle
435 of Southern China. J. Parasitol. 97, 172–173.
436 Ybañez, R.H., Terkawi, M.A., Kameyama, K., Xuan, X., Nishikawa, Y., 2013. Identification
437 of a highly antigenic region of subtilisin-like serine protease 1 for serodiagnosis of
438 *Neospora caninum* infection. Clin.Vaccine. Immunol. 20, 1617-1622.
439 Zhou, D.H., Zhao, F.R., Lu, P., Xia, H.Y., Xu, M.J., Yuan, L.G., Yan, C., Huang, S.Y., Li, S.J.,
440 Zhu, X.Q., 2012. Seroprevalence of *Toxoplasma gondii* infection in dairy cattle in
441 southern China. Parasit. Vectors 5:48.

442 **Table titles**

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

445 **Table 2.** Seroprevalence of *T. gondii* in different regions of Mongolia.

446 **Table 3.** Analysis of risk factors associated with *T. gondii* infection in Mongolian cattle.

447 **Table 4.** Seroprevalence of *N. caninum* in different regions of Mongolia.

448 **Table 5.** Analysis of risk factors associated with *N. caninum* in Mongolian cattle.

449

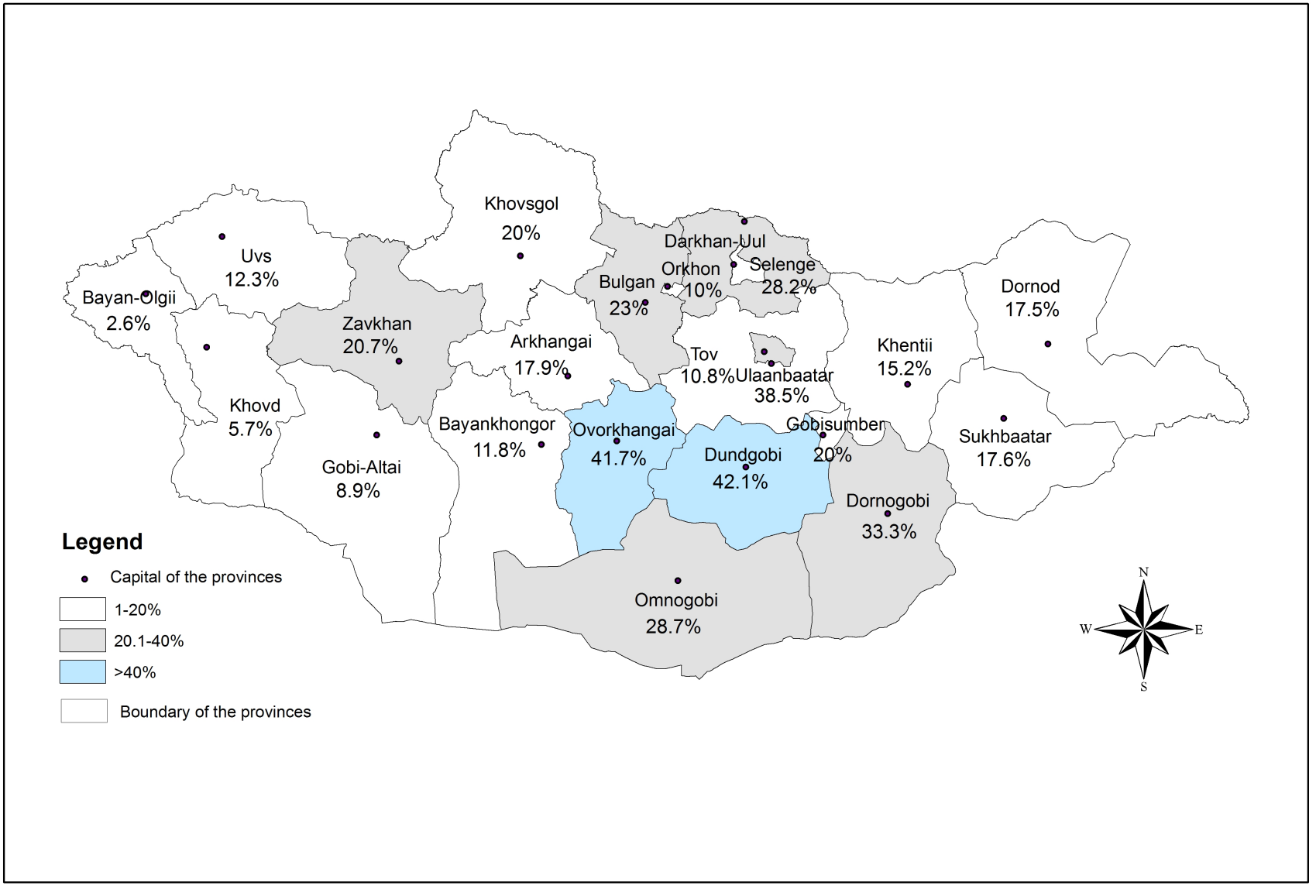
450 **Figure legends**

451 **Fig. 1.** Geographical distribution of *T. gondii* infection in Mongolian cattle used in this study.
452 Infection rates are indicated as follows: white 1%–20%, gray 20.1%–40%, and light blue
453 >40%.

454

455 **Fig. 2.** Geographical distribution of *N. caninum* infection in Mongolian cattle used in this study.
456 Infection rates are indicated as follows: white 1%–20%, gray 20.1%–40%, and light blue
457 >40%.

458


Khovsgol
20%
Uvs
12.3%
Darkhan-Uul
Orkhon
10%
Selenge
28.2%
Bulgan
23%
Bayan-Olgii
2.6%
Zavkhan
20.7%
Arkhangai
17.9%
Tov
10.8%
Ulaanbaatar
38.5%
Khentii
15.2%
Dornod
17.5%
Khovd
5.7%
Bayankhongor
11.8%
Ovorkhangai
41.7%
Gobisumber
20%
Dundgobi
42.1%
Sukhbaatar
17.6%
Gobi-Altai
8.9%
Dornogobi
33.3%
Omnogobi
28.7%
Legend
Capital of the provinces
1-20%
20.1-40%
>40%
Boundary of the provinces
N
W
E
S

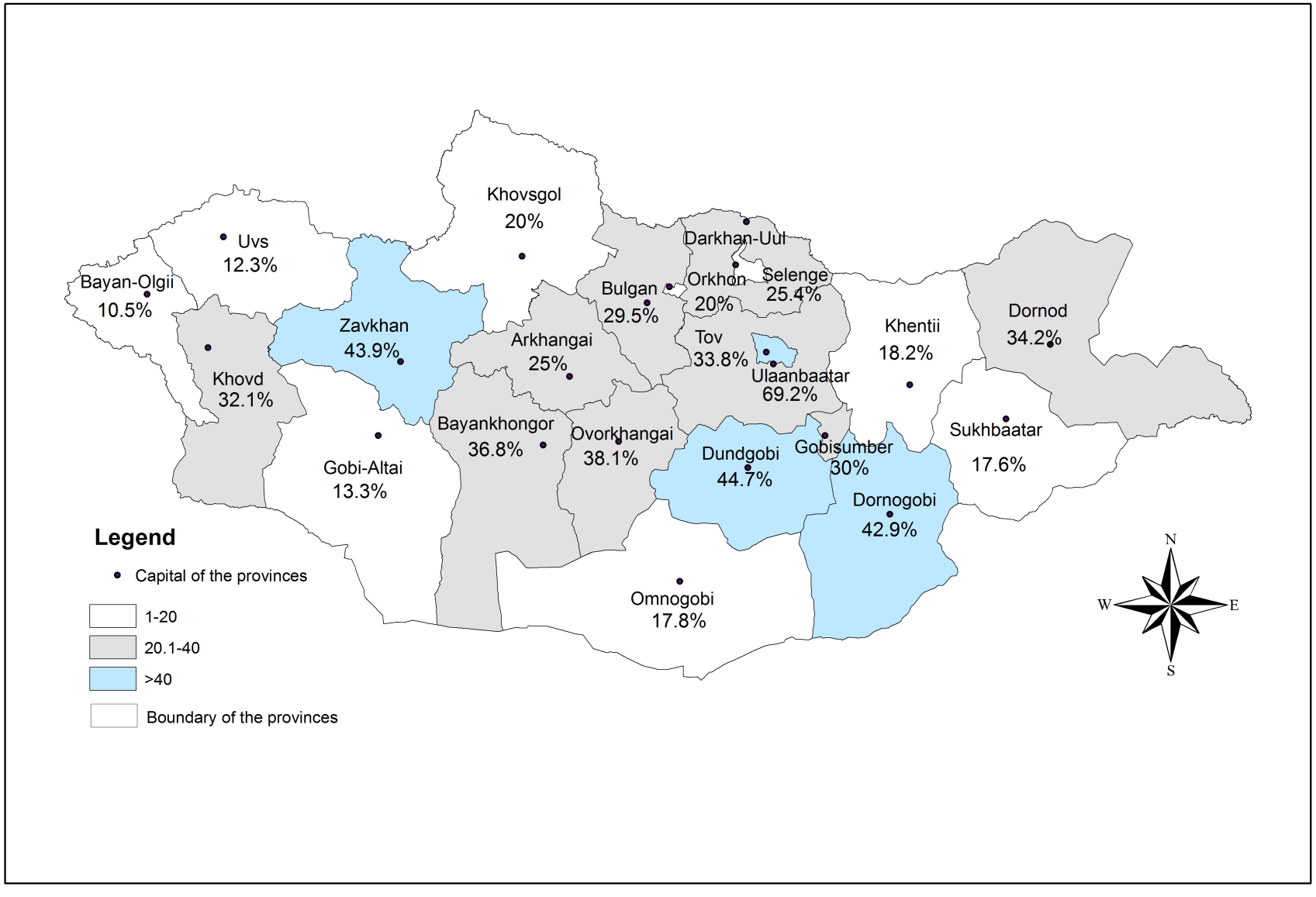

Khovsgol
20%
Uvs
12.3%
Darkhan-Uul
Bayan-Olgii
10.5%
Orkhon
20%
Selenge
25.4%
Bulgan
29.5%
Zavkhan
43.9%
Arkhangai
25%
Tov
33.8%
Khentii
18.2%
Dornod
34.2%
Khovd
32.1%
Ulaanbaatar
69.2%
Bayankhongor
36.8%
Ovorkhangai
38.1%
Sukhbaatar
17.6%
Dundgobi
44.7%
Gobisumber
30%
Gobi-Altai
13.3%
Dornogobi
42.9%
Omnogobi
17.8%
Legend
Capital of the provinces
1-20
20.1-40
>40
Boundary of the provinces
N
W
E
S

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 (269/1,438)	16.7-20.8	26.2 (377/1,438)	23.9-28.5	6.8 (98/1438)	5.6-8.2

CI: confidence interval.

Table 2

Seroprevalence of *T. gondii* in different regions of Mongolia

Regions of Mongolia	No. tested	No. positive	No. negative	Seroprevalence (%)	OR (95%CI)	*P*-value
Central region	350	95	255	27.1	0.26 (0.17-0.40)	<0.0001
Western region	398	36	362	9	-	
Eastern region	348	58	290	16.6	0.49 (0.31-0.77)	0.002
Khangai region	342	80	262	23.3	0.32 (0.1-0.49)	<0.0001

OR: odds ratio, CI: confidence interval, -: reference group.

Table 3

Analysis of risk factors associated with *T. gondii* infection in Mongolian cattle

Risk factors		No. sampled	No. positive	No. negative	Seroprevalence (%)	OR(95%CI)	*P*-value
Age groups	1--4	710	135	575	19	-	
(Years)	5--9	430	75	355	17.4	1.11 (0.81-1.51)	0.507
	10--14	44	10	34	22.7	0.79 (0.38-1.65)	0.543
	Unknown	254	49	205	19.2	0.98 (0.68-1.41)	0.92
Sex	Female	1,174	213	961	18.1	-	
	Male	264	56	208	21.2	0.82 (0.59-1.14)	0.248

OR: odd ratio, CI: confidence interval, - : reference group.

Table 4

Seroprevalence of *N. caninum* in different regions of Mongolia

Regions of Mongolia	No. tested	No. positive	No. negative	seroprevalence (%)	OR (95%CI)	*P*-value
Central region	350	108	242	30.8	0.59 (0.42-0.82)	0.002
Western region	398	83	315	20.8	-	
Eastern region	348	81	267	23.2	0.86 (0.61-1.22)	0.427
Khangai region	342	105	237	30.7	0.59 (0.42-0.83)	0.002

OR: odds ratio, CI: confidence interval, - : reference group.