Title:

Seroepidemiological study of *Toxoplama gondii* in small ruminants (sheep and goat) in different provinces of Mongolia

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Abstract

Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii*. Consumption of raw or undercooked meat is the main risk factor for acquiring *T. gondii* infection in humans. Meat and meat products derived from goats and sheep are mainly consumed in Mongolia; however, there is limited epidemiological information on *T. gondii* infection in small ruminants in this country. The main objective of the present study was to investigate the seroprevalence of *T. gondii* in sheep and goats in Mongolia. The seroprevalence of *T. gondii* IgG antibodies was determined by an indirect enzyme-linked immunosorbent assay based on the recombinant antigens of dense granule protein 7 of *T. gondii*. A total of 1,078 goat and 882 sheep blood samples were collected from 17 of 21 provinces and the capital city of Mongolia. Overall, the seroprevalence of *T. gondii* among the goat and sheep samples was 32% and 34.8%, respectively. The seroprevalence among goat samples was significantly higher in western (42.7%) and eastern (45.6%) regions compared with other regions (24%). Additionally, the seroprevalence among sheep was significantly higher in eastern regions (55.4%) compared with other regions (26%–33%). Age, but not sex, was considered a risk factor for *T. gondii* seropositivity in goats, whereas no statistically significant differences were observed in sheep for age or sex. In conclusion, the present study demonstrates the high seroprevalence of *T. gondii* in small ruminants in Mongolia. Our results highlight that country-wide control measures are required to minimize infections in livestock.

Keywords: *Toxoplasma gondii*; Seroprevalence; Goat; Sheep; TgGRA7; Mongolia.

1. Introduction

Toxoplasmosis is a disease that occurs worldwide and is caused by the intracellular apicomplexan protozoan parasite *Toxoplasma gondii*. *T*. *gondii* is capable of infecting almost all types of animals including humans, livestock, and wild animals. Small ruminants (sheep, goats) and cattle are the intermediate hosts of *T*. *gondii*. Domestic cats and wild felids are the definitive hosts that can excrete oocysts into the environment [1].

Previous studies indicated that ingestion of oocytes through food and water is the main mode of transmission of *T*. *gondii* in humans [2]. Animals and humans can become infected by ingesting tissue cysts from undercooked meat or soil, or water contaminated with infective oocysts [3]. *T*. *gondii* tissue cysts are commonly observed in food-producing animals including pigs, chickens, sheep, and goats [4]. Sheep and goats are more susceptible to *T*. *gondii* infection. Abortion and neonatal infections in sheep and goats occur as a result of primary infection during pregnancy [5].

Many studies have been conducted to investigate the seroprevalence of *T*. *gondii* in small ruminants in different areas of the world [6]. However, toxoplasmosis has not been well-studied among livestock in Mongolia. In a previous study, the seroprevalence of *T*. *gondii* among sheep in Mongolia was estimated through an indirect enzyme-linked immunosorbent assay (iELISA) based on recombinant *T*. *gondii* matrix antigen 1 and a latex agglutination test (LAT). The overall seroprevalence rate of *T*. *gondii* was 24% (42/175) by iELISA and 16% (29/175) by LAT [7]. Furthermore, the seroprevalence of *T*. *gondii* in wild Pallas' cats, which is a small-sized felid species (*Otocolobus manul*), was 13% (2/15) [8]. In our previous study, the seroprevalence of *T*. *gondii* among cattle in Mongolia was examined using an iELISA based on recombinant antigen TgGRA7. A total of 1,438 cattle sera from 20 of the 21 provinces of Mongolia and the capital city of Ulaanbaatar were evaluated. According to this study, 18.7%

of cattle were seropositive for specific antibodies to *T. gondii* [9], confirming the presence of *T. gondii* in Mongolia.

In Mongolia, there is only one report documenting the seroprevalence of *T. gondii* in sheep, but none for domestic goats. Information on the seroprevalence of *T. gondii* in small ruminants such as goats and sheep will be helpful in implementing preventive strategies for public health. Therefore, the main objectives of this survey were to investigate the seroprevalence of *T. gondii* in small ruminants (sheep and goats) from different areas in Mongolia and to evaluate the risk factors associated with seropositivity.

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

Blood samples were taken from 1,078 goats and 882 sheep in 17 of 21 provinces of Mongolia during 2016–2018. The sheep and goats are grazed together based on nomadic lifestyle. Mongolia is divided into four major regions comprising 21 provinces: the central region (Tov, Omnogobi, Gobisumber, Dornogobi, Selenge, and Dundgobi), the western region (Gobi-Altai, Khovd, Bayan-Olgii, Uvs, and Zavkhan), the eastern region (Sukhbaatar, Dornod,

and Khentii), and the khangai region (Khovsgol, Arkhangai, Ovorkhangai, Bayankhongor, Bulgan, and Orknon).

2.3. Sample collection

Approximately 2 ml of blood was taken from each animal (goats and sheep) by venal puncture into glass tubes containing anticoagulant (EDTA). All samples were kept in an icebox and transferred to the Laboratory of Molecular Genetics, at the Institute of Veterinary Medicine, Mongolian University of Life Sciences. Then blood samples were centrifuged to collect the sera and the sera were kept frozen at -30°C until analysis.

2.4. Recombinant protein expression and purification of soluble protein TgGRA7

Recombinant TgGRA7 (rTgGRA7) was expressed as glutathione S-transferase (GST) fusion protein in *E. coli* DH5α (Takara Bio, Inc., Japan) using previously described methods [10]. The glutathione *S*-transferase (GST)-tagged recombinant protein was separated by thrombin protease in accordance with the manufacturer's instructions (GE Healthcare, Buckinghamshire, UK). The expression of rTgGRA7 (29 kDa) was then confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie brilliant blue R250 staining (MP Biomedicals, Inc., France). The rTgRGA7 protein concentration was subsequently measured using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc., Rockford, IL, USA).

2.5. iELISA

Fifty microliters of purified antigen at a final concentration of 0.1 µM were added along with coating buffer (carbonate-bicarbonate buffer; Sigma, St. Louis, MO, USA) to the wells of an ELISA plate (Nunc, Roskilde, Denmark) and the plate was incubated overnight at 4°C. After washing with phosphate-buffered saline (PBS: 0.05%, Tween 20), the plate was blocked with 100 µl of blocking solution containing 3% skimmed milk in PBS (PBS-SM) for 1 h at 37°C. After one further wash with washing buffer, goat and sheep sera were diluted 1:200 with 3% skim milk in PBS, and then 50 µl of positive control, negative control, and test serum samples 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-conjugated anti-sheep IgG (Bethyl Laboratories, Montgomery, TX, USA) diluted 1:5,000 and anti-goat IgG (Bethyl Laboratories) diluted 1:10,000 (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 substrate solution were quickly added to each well and incubated at room temperature for 1 h in the dark. The absorbance values at 415 nm (A_{415}) 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_{415} value for negative goat sera (n = 9 negative sera) and negative sheep sera (n = 9 negative sera) plus three standard deviations. The reference negative sera used for *Toxoplasma* studies were confirmed by both a commercial latex agglutination test (Toxocheck-MT, Eiken Chemical, Tokyo, Japan) and a TgGRA7-based iELISA. The positive control sera of goat (n = 3) and sheep (n = 3) used in the present study were confirmed by the commercial latex agglutination test and TgGRA7 based iELISA.

2.6. Statistical analysis

Data analysis was performed using GraphPad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA). The Chi-square test was used to analyze data. Associations were tested using odds ratios (ORs) and 95% confidence intervals (CIs) after adjustment. A *P* value of < 0.05 was considered statistically significant using the VassarStats online tool (http://vassarstats.net/).

3. Results

The sera from goats (n=1,078) and sheep (n=882) were collected and tested in 17 of 21 provinces, and the capital city of Ulaanbaatar in Mongolia. The overall seroprevalence of *T. gondii* in goats was 32% (345/1078) ranging from 0% to 65% (Table 1). Although no correlation was found with sex, age was associated with seroprevalence in goats, with higher seroprevalence being observed in 1–2-year-old (39.7%) and >7-year-old animals (36.7%) (Table 2). Furthermore, geographical location was identified as a risk factor in goats (Table 2). The highest seroprevalence was identified in goats in the eastern and western regions of Mongolia (45.6% and 42.7%, respectively). In particular, higher rates were observed in Uvs and Zavkhan provinces in the western region (61.3% and 65.0%, respectively), (Table 1).

The overall seroprevalence of *T. gondii* in sheep was 34.8% (307/882) ranging from 10.5% to 80.5% (Table 3). There was no correlation detected between seroprevalence and the age or sex of animals (Table 4). However, geographical differences were detected with high seroprevalence observed in the eastern region of Mongolia (55.4%) ($P < 0.0001$) (Table 4). The highest seroprevalence was identified in Khentii province, in the eastern region of Mongolia, with a prevalence of 80.5%, followed by Umnugobi province, in the central region, with a prevalence of 61.3%.

4. Discussion

The livestock industry plays a crucial role in the economy of Mongolia. The total livestock population, comprising cattle, sheep, goats, camels, and horses, was 66 million in 2018 [11]. Among them, cattle, sheep, and goats are the main sources of food for Mongolians and meat products derived from sheep and goats are a daily part of many peoples' diet. Recently, the goat and sheep populations have reached 41.6%–45.4% of the total livestock. The most consumed meats are mutton (31.5%), chevon (27.7%), and beef (21.3%) in Mongolia [12].

Toxoplasmosis has been identified as a foodborne disease of global concern [13]. Due to increased awareness to food safety, monitoring of pathogen infection in livestock needs to be implemented in Mongolia. In this study, the seroprevalence of *T. gondii* antibodies in sheep and goats in Mongolia was examined using TgGRA7-iELISA. TgGRA7 has been reported to be a useful diagnostic marker for the detection of IgG antibodies in acute and chronic infections with higher potency, specificity, and sensitivity [10, 14, 15]. Sheep and goats are highly susceptible to *T. gondii* infection, which plays a major role in the transmission of toxoplasmosis into humans [16]. Raw or undercooked meat from these animals is potentially hazardous if consumed by humans or other animals. The high seroprevalence of *T. gondii* in Mongolia in this study may present a risk of human infection in the country. Drinking the oocyst-contaminated water, consumption of unwashed vegetables and fruits, as well as direct environmental contamination, can cause *T. gondii* infection in humans [17]. Additionally, *T. gondii* DNA was detected in the milk from one sheep and eight Bactrian camels in Mongolia [18]. Although Mongolians typically do not consume undercooked meat, or unpasteurized milk from livestock, these high risk foods are still potential risk factors for *T. gondii* prevalence among Mongolians.

China and the Russian Federation are the only neighboring countries of Mongolia. In China, the seroprevalence of *T. gondii* in sheep ranged from 0.8% to 39.3% in different areas [19], but information on the prevalence in goats is limited [20]. In the Russian Federation, antibodies to *T. gondii* were detected in goats (43.9%), cats (39.9%), and humans (30.9%) [21]. The overall seroprevalence of *T. gondii* in sheep (34.8%) and goats (32%) in Mongolia obtained in this study is similar that of neighboring countries. Previous studies have shown an association between *T. gondii* seropositivity and identified risk factors in ruminants including animal age, environmental conditions, breed, the presence of cats, and rodent control [22,23,24]. In the present study, only geographical location had a significant association with *T. gondii* seropositivity for both sheep and goats. Variations in the prevalence obtained per area in this study may be due to the differences in climate and environmental conditions of the sampling locations.

Mongolia has an extreme climate that is highly variable between regions. The country has four seasons with large temperature fluctuations and low precipitation. A previous study reported the differing prevalence of *T. gondii* in areas with varying altitudes. In the case of school children in Panama, lower prevalence was seen in the areas of highest altitude, while higher prevalence was observed in areas near the sea [25]. In the present study, significantly higher seroprevalence of *T. gondii* was detected in goats in the eastern region (45.5%), which can be attributed to the lower altitude of this region. Moreover, the highest seroprevalence of *T. gondii* in sheep was observed in the eastern region of Mongolia (55.4%).

The climatic characteristics of the eastern region may explain the higher *T. gondii* seroprevalence found in this region. The eastern region of Mongolia is characterized by steppes with a relatively high annual precipitation (180–280 mm) [26]. This high level of precipitation may make the spread of oocysts easier, which may contribute to the higher seroprevalence of *T. gondii* in the eastern region.

Moreover, we found higher seroprevalence of *T. gondii* in sheep and goats of 1–2 years of age compared with 3–4 and 5–6-year-old animals, and this difference was statistically significant for goats. These results indicate that these animals were exposed to *T. gondii* at a young age. Because winter conditions, particularly heavy snow and/or low temperatures, cause serious damage to livestock in Mongolia, *T. gondii* infection might decrease the survival of young animals. Therefore, the seroprevalence in animals over 3 years of age might be lower than in 1–2-year-old animals.

Some species of wild cats such as *Felis silvestris* (wild cat), *Lynx lynx* (Euasian lynx), *O. manul* (Pallas' cat), and *Uncia uncia* (snow leopard) are distributed across Mongolia. The Pallas' cat and snow leopard inhabit the Huvsgul mountain range and the Mongol Altai mountain range, which are located in the western region of Mongolia [28]. These wild cat species might be potential source of infection. This distribution is consistent with the significantly higher seroprevalence of *T. gondii* observed in goats in the western regions of Mongolia (42.6%). Domestic cat population is not widespread in the country and Mongolian herders do not regularly keep cats as domestic pets, lowering the chance of contact with infected cat feces. However, some domestic cats are kept and even shared among households to control rodents. In this study, we found similar seropositive rates of *T. gondii* in goats (32%) and sheep (34.8%). This may be attributed to the fact that all livestock are fed by grazing on open pastures and river water, and several animals are herded together under the extensive livestock system. This extensive livestock system where all the animals especially sheep and goats are grazed on the natural grassland together. Environmental condition is Mongolia characterized by harsh climate and grasslands are not similar with the whole area, thus Mongolian animal husbandry based on nomadic lifestyles.

Statistics have been reported on the number of livestock that died of disease in 2017: sheep 53%, goats 26.8%, cattle 16.6%, horses 3.5%, and camels 0.1% [11]. In 2017, the

Mongolian National Statistical Office reported that the rate of abortion in livestock was 44.6% in the khangai region, 25.7% in the central region, 14.7% in the western region, and 14.0% in the eastern region [29] which might be caused by parasitic infections. In our present study, a higher seroprevalence of *T. gondii* infection was confirmed in goats and sheep, indicating that they are susceptible hosts of *T. gondii*. This higher seroprevalence of *T. gondii* might affect livestock mortality rates.

In conclusion, our data may be useful in developing and improving prevention and control strategies for the management of toxoplasmosis in livestock in Mongolia. Our data may also be helpful in determining whether domestic and wild cats shed oocysts in provinces that show higher seroprevalence. Further studies to assess the impact of *T. gondii* infection in humans are required in Mongolia.

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Conflict of interests

257 The authors declare that they have no conflict of interest.

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336

337 **Figure legends**

338 **Fig. 1.** Geographical distribution of *T*. *gondii* in Mongolian goats included in this study.
339 Infection rates are indicated as follows: white 0%–30%, gray 30.1%–50%, and light blue
340 >50%.

341

342 **Fig. 2.** Geographical distribution of *T*. *gondii* in Mongolian sheep included in this study.
343 Infection rates are indicated as follows: white 0%–30%, gray 30.1%–50%, and light blue
344 >50%.

345

Figure 1

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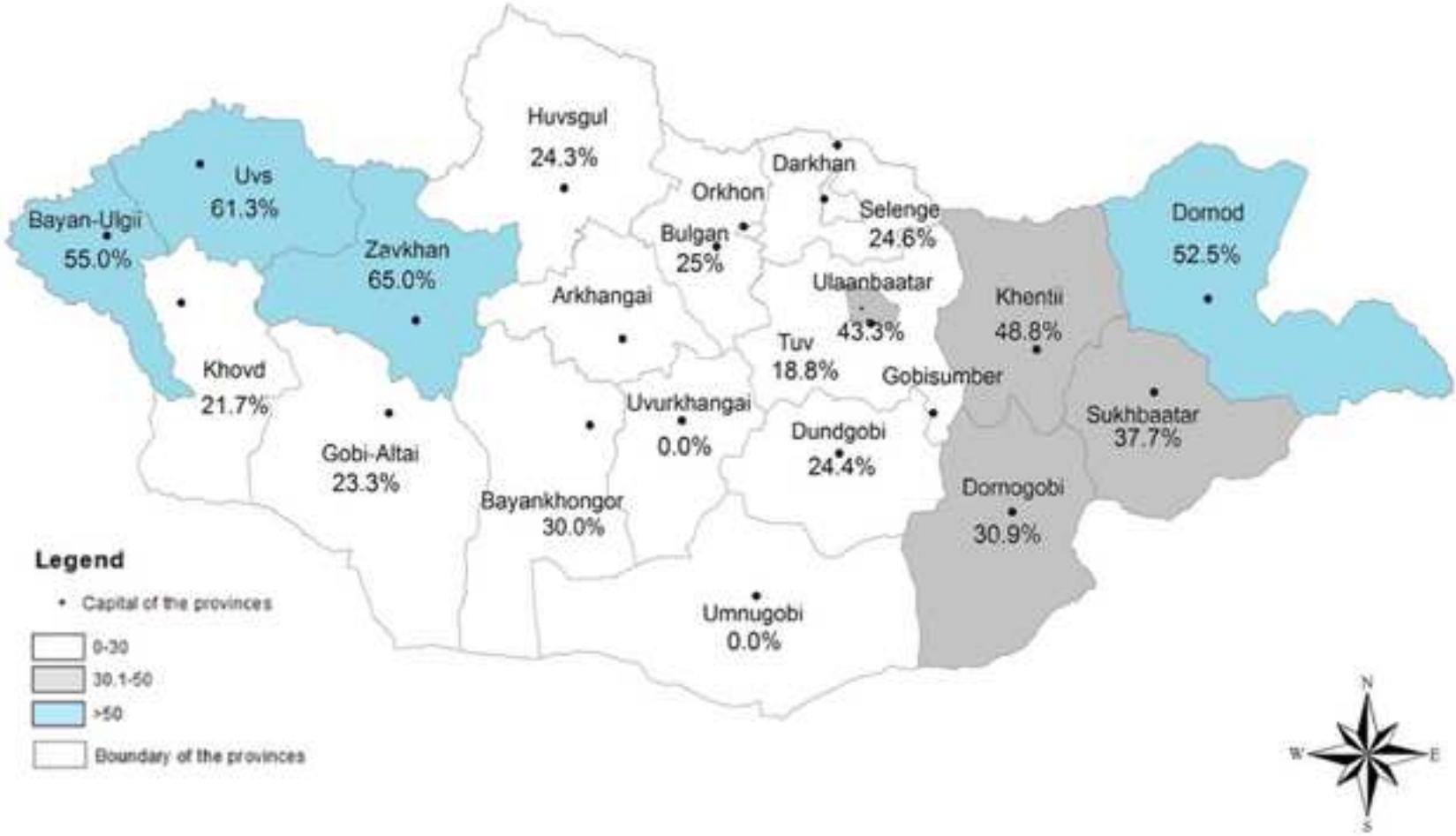

Figure 2

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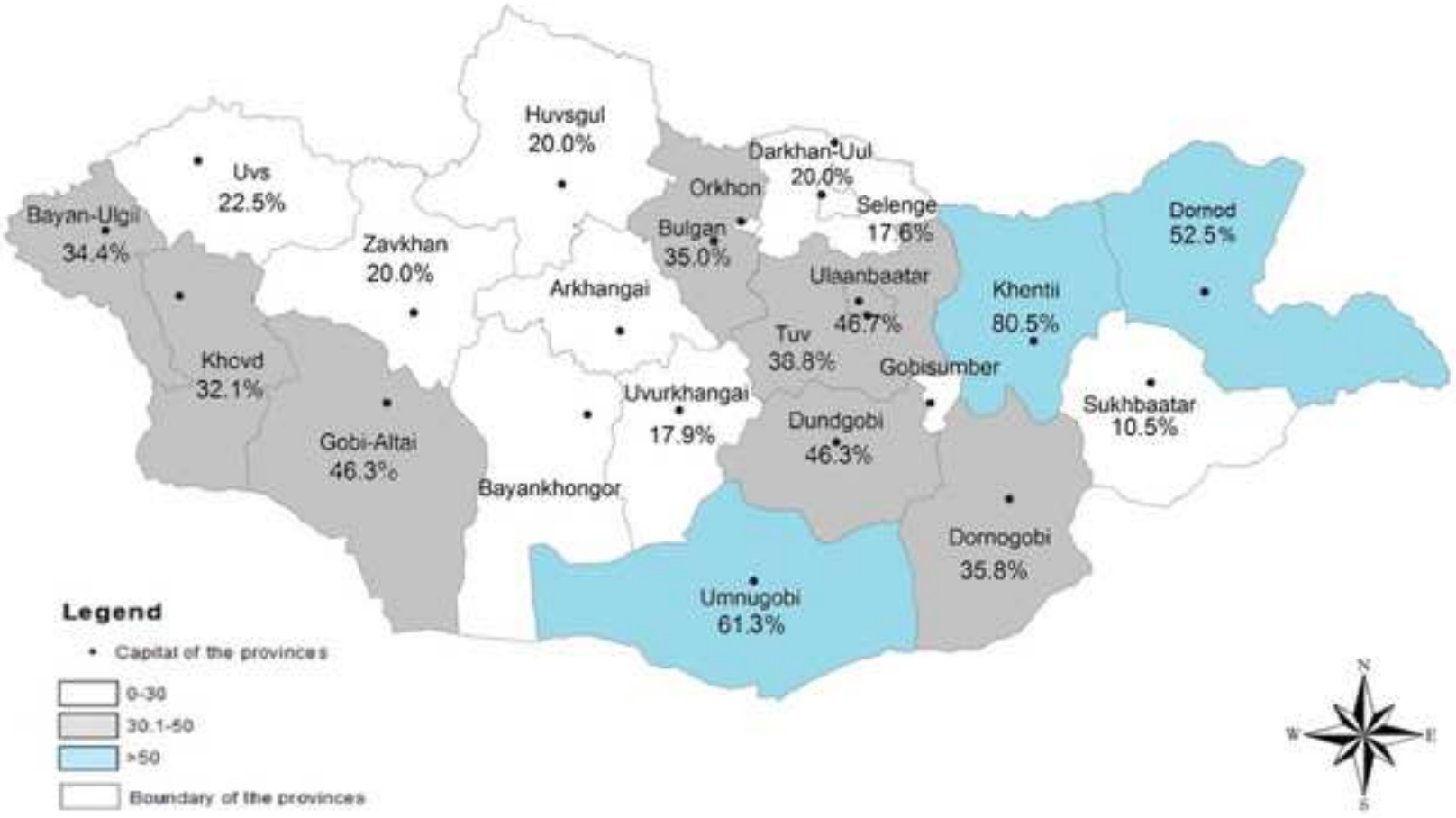

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