SEROPREVALENCE OF OVINE TOXOPLASMOSIS IN MONGOLIAN SAMPLES USING AN ELISA WITH RECOMBINANT *TOXOPLASMA GONDII* MATRIX ANTIGEN 1

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ABSTRACT

The seroprevalence of T.gondii infection in Mongolian sheep was investigated using an ELISA with rTgMAG1. A total of 175 serum samples collected from seven aimags in Mongolia were examined by an ELISA, and the results were compared with those from the commercialized LAT. Although, both tests showed high concordance, the ELISA detected 24% of infection and it seems to be more sensitive than the LAT detected 16.5% of infection in ovine samples. These results emphasize the usefulness of rTgMAG1 for diagnostic use in detection of T.gondii infection.

KEY WORDS: *Toxoplasma gondii,* TgMAG1, *Toxoplasma gondii* matrix antigen 1

BACKGROUND

Toxoplasma gondii is an obligate intracellular protozoan parasite, belongs to the phylum Apicomplexa that is capable of infecting a broad range of hosts, including humans and domestic animals [1]. *Toxoplasma gondii* is an important cause of abortion and neonatal mortality in sheep and goats in many countries [2;3]. Most sheep acquire *T gondii* infection after birth, and less than 4% of persistently infected sheep transmit the parasite vertically to the next generation [4;5;6]. The infection to humans occurs after ingesting tissue cysts from undercooked lamb and consuming food or drink contaminated with oocysts [7;2;8;3].

Livestock production is the most important economic resource in Mongolia, depends on nomadic animal husbandry. Due to the facts that sheep and their products represent the major source of meat and milk for habitants in Mongolia coupled with the lack of sufficient data about the incidence of ovine toxoplasmosis in sheep, it is particularly beneficial to ensure the prevalence of this parasite in sheep destined for human consumption.

Therefore, the present study investigates the seroprevalence of *T.gondii* infection in sheep in Mongolia using enzyme-linked immunosorbent assay with recombinant TgMAG1.

MATERIAL AND METHODS

Expression and purification of recombinant TgMAG1. Recombinant TgMAG1 was expressed as the GST-fusion protein. The protein was purified using glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech, USA). After dialysis and filtration, the purity of the purified protein was estimated by SDS-PAGE.

ELISA. Ninety-six-well plates (Nunc, Denmark) were coated with rTgMAG1 (1 μg/ml) and GST (1 μg/ml) diluted in the antigen coating buffer (0.05 M carbonate buffer, pH 9.6) at 4^{o}C overnight. The plates were washed with washing solution (PBS containing 0.05% Tween 20) and then the wells were blocked with blocking solution (PBS containing 3% skim milk) at 37^{o}C for 1 hr. After discarding the blocking solution the wells were incubated with the test sera (1:100) at 37^{o}C for 1 hr. After washing the wells were incubated with horse radish peroxidase-conjugated anti-sheep IgG antibody (Bethyl, USA; 1:4,000) at 37^{o}C for 1 hr. After washing the wells were incubated with substrate solution [0.1 M citric acid, 0.2 M sodium phosphate, 0.003% H_2O_2, 0.5 mg of 2,2'-azinobis (3-ethylbenzthiazoline sulfonic acid) per ml] at room temperature for 1 hr. The optical density (OD) was measured with the MTP-500 microplate reader (Corona Electric, Japan) at 415 nm. The positive cut-off value was calculated as the mean OD value of the 30 serum samples from IFAT-negative animals plus 3-fold of their standard deviation.

Latex agglutination test. The latex agglutination test (LAT) was performed according to the manufacturer's instruction (Toxocheck-MT; Eiken Chemical, Japan). It was considered positive when agglutination was observed at dilutions of 1:32 and greater.

Serum samples. A total of 175 serum samples were collected from sheep in Selenge (n=25), Tuv (n=25), Hovd (n=25), Uvs (n=25), Dornod (n=25), Bulgan (n=25), and Sukhbaatar (n=25) aimags in Mongolia (Fig. 6). The samples were from female and males animals that are 1-8 years old. The samples were divided into three groups based on the age of sheep: A, age ranged between 1 to 2 years; B - 3-5 years; C - sheep above 5 years old.

RESULTS

A total of 175 samples collected from sheep in different aimags in Mongolia were tested for the detection of antibodies to *T.gondii* infection by the ELISA with rTgMAG1, and the results were compared with those from the commercialized LAT.

As shown in Table 1, of 175 serum samples analyzed, 42 (24%) and 29 (16.57%) samples were positive by the ELISA and LAT, respectively. Notably, out of 29 LAT-positive samples, 27 were positive by the ELISA, revealing high concordances between these tests (93.10%). On the other hand, positive rates of the ELISA among 7 aimags ranged from 12% detected in Hovd province to 36% detected in Selenge aimag (Table 2). Thereafter, the statistical analyses of risk factors associated with the seropositive ELISA were investigated. The positive rate of the ELISA was 25.9% in female and 20.3% in male sheep (Table 3) and these were not significantly different (P> 0.05). In contrast, a significant difference was in positive rates of the ELISA among different age groups that ranged from 8.7% (1-2 years) to 26.1% (>6 year) as shown in Table 4 (P< 0.05).

Table 1

Seroprevalence of ovine toxoplasmosis in Mongolia as determined by the ELISA with rTgMAG1 and LAT

LAT[b]	ELISA[a]		Total
	+	-	
+	27 (15%)	2 (1.14%)	29 (16.57%)
-	15 (8.57%)	131 (74.86%)	146 (83.43%)
Total	42 (24%)	133 (76%)	175 (100%)

a) ELISA was considered positive when an OD at 415 nm of 0.2 was observed at dilution of 1:100 greater.
b) LAT was considered positive when agglutination was observed at dilutions of 1:32 and greater.

Table 2

Seroprevalence of ovine toxoplasmosis in 7 aimags as determined by the ELISA with rTgMAG1 and LAT

Aimag	No. of sample	ELISA[a]		LAT[b]	
		+	-	+	-
Selenge	25	9 (36%)	16 (64%)	9 (36%)	16 (64%)
Tuv	25	7 (28%)	18 (72%)	6 (24%)	19 (76%)
Hovd	25	3 (12%)	22 (88%)	3 (12%)	22 (88%)
Uvs	25	5 (20%)	20 (80%)	5 (20%)	20 (80%)
Dornod	25	4 (16%)	21 (84%)	3 (12%)	22 (88%)
Bulgan	25	7 (28%)	18 (72%)	2 (8%)	32 (92%)
Sukhbaatar	25	7 (28%)	18 (72%)	1 (4%)	24 (96%)
Total	175	42 (24%)	133 (76%)	29 (16.57%)	146 (83.43%)

a) ELISA with rTgMAG1 antigen.

b) LAT was performed according to manufacturer's instruction (Toxocheck-MT, Eiken Chemical, JAPAN).

Table 3

Seroprevalence of ovine toxoplasmosis in 7 aimags as determined by the ELISA with rTgMAG1 in different gender groups

Gender	No. of examined	No. of positive	No. of negative
Female	116	30 (25.9%)	86 (74.1%)
Male	59	12 (20.3%)	47 (79.7%)
Total	175	42 (24%)	133 (76%)

Table 4

Seroprevalence of ovine toxoplasmosis in 7 aimags as determined by the ELISA with rTgMAG1 in different age groups.

Age	No. of examined	No. of positive	No. of negative
Young, 1-2	23	2 (8.7%)	21 (91.3%)
Middle, 3-5	129	34 (26.35%)	95 (73.65%)
Above 6	23	6 (26.08%)	17 (73.92%)
Total	175	42 (24%)	133 (76%)

DISCUSSION

Toxoplasmosis is of great importance worldwide because it causes abortions, stillbirth, and neonatal loss in all types of livestock, especially in sheep and goats [2]. In addition, the tissue cysts of *T.gondii* in meat of infected livestock are in important source of infection humans [19]. Sheep are an important source of meat and milk to inhabitants in Mongolia, and the ingestion of these products might pose risks to public health. However, there is no data about the incidence of ovine toxoplasmosis in sheep in Mongolia. Therefore, I have aimed in this study to develop diagnostic test using recombinant TgMAG1 to investigate the seroprevalence of *T. gondii* infection in Mongolian sheep.

The high specificity and sensitivity of rTgMAG1 in the detection of infection in mouse models, combined with its reportedly diagnostic potential in the detection of human toxoplasmosis [17] were motivating to examine the usefulness of this antigen for detection of ovine toxoplasmosis. Sheep sera collected from different aimags in Mongolia were examined for the presence of a specific antibody to *T.gondii* by the ELISA with rTgMAG1, and the results were compared with those from the commercialized LAT.

A total of 175 serum samples analyzed, 42 (24.00%) and 29 (16.57%) samples were positive by the ELISA and LAT, respectively. These results revealed the high sensitivity of the ELISA in the detection of ovine toxoplasmosis. The higher positive proportion detected by the ELISA than by the LAT can be explained by the abundant expression of rTgMAG1 in tachyzoites and bradyzoites stages, while only soluble tachyzoite antigens are used in the LAT.

The prevalence of toxoplasmosis across the world is variable depending upon the country's customs, traditions, the lifestyles of the inhabitants, climatic variations, the age of the animals, and the husbandry methods, which are essential elements in

epidemiological studies [27]. The seroprevalence of ovine toxoplasmosis has been reported from many countries: Canada (57%), Turkey (55%), Poland (53%), Bulgaria (48%), Iran (35%), Morocco (27%), Greece (23%), and Brazil (18%) [20;21;22;23;24;25;26;27].

The prevalence rate of sheep samples by the ELISA with rTgMAG1 was determined on the basis of different genders, ages and geographical regions. The statistical analyses of risk factors associated with seropositive ELISA were investigated and revealed that sheep sampled from Selenge aimag had the highest prevalence 36%. Moreover, the prevalence was significantly increased in aged sheep. Taken together, these results indicate the widespread *T. gondii* in sheep, which may pose a potential source of infection for human through the consumption of their products.

CONCLUSION

LAT and ELISA results demonstrate the successful use of rTgMAG1 as a reagent for detection of the infection and indicate the widespread of *T.gondii* infection in sheep in Mongolia.

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