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Seroprevalence of *Toxoplasma gondii* infection in sheep from northern China

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Abstract. *Toxoplasma gondii* is an important zoonotic parasite causing significant health problems to humans and animals. In recent years, a number of investigations about the seroprevalence of *T. gondii* in China have been reported, but little is known on the prevalence of toxoplasmosis in sheep in northern China. In the present study, a total of 288 sheep serum samples were collected from Inner Mongolia, Heilongjiang, Jilin and Hebei provinces of northern China for *T. gondii* antibody survey using a latex agglutination test (LAT). Of these, 87 (30.2%) serum samples were positive for antibodies to *T. gondii*, and the antibody titres ranged from 1:64 to 1:1,024. Seroprevalence of *T. gondii* infection in sheep was 17.1% in Inner Mongolia, 33.8% in Heilongjiang, 24.6% in Jilin and 46.3% in Hebei. Age and rearing system significantly affected seropositivity. The present survey indicates antibodies to *T. gondii* are widely prevalent in sheep in northern China, which may cause public health problems in these provinces.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite with a wide range of intermediate hosts including humans, while felids serve as definitive hosts and produce the environmentally resistant oocysts (Dubey 2010). Toxoplasmosis, an important zoonotic disease caused by *T. gondii*, elicits psychiatric disorders, abortion and foetal abnormality in pregnant women or animals and continues to be a major public health problem (Montaya & Liesenfeld 2004; Hill & Dubey 2015; Shiadeh *et al*., 2017). *T. gondii* can infect animals and humans via three main pathways: ingestion of oocysts from the environment, ingestion of tissue cysts in undercooked meat, and congenital transmission from an infected mother to the foetus during pregnancy (Montaya & Liesenfeld 2004; Dubey 2010). Many people have the habit of eating under-cooked 'barbecue' and 'instantly boiled mutton' in China. Ingestion of raw or undercooked meat of animals harbouring tissue cysts is considered the most important source of human infection (Hill & Dubey, 2015). Thus, humans can get infected with *T. gondii* by ingestion of undercooked lamb and mutton.

In recent years, a growing number of surveys of *T. gondii* infection in sheep have been carried out worldwide (Gebremedhin *et al*., 2014; Hammond-Aryee *et al*., 2015; Tegegne *et al*., 2016; Özmutlu & Karatepe., 2017), and several surveys have shown that *T. gondii* infection in sheep is common in some provinces of China (Wu *et al*., 2011; Wang *et al*., 2011; Xu *et al*., 2015; Liu *et al*., 2015; Yin *et al*., 2015; Zou *et al*., 2015). However, there is a gap in knowledge of *T.*

gondii infection in sheep in northern China. Considering the public health importance and economic significance of products from sheep, it is essential to comprehensively understand the occurrence of *T. gondii* infection in sheep. Therefore, we performed a survey on the seroprevalence of *T. gondii* infection in sheep from northern China and to assess risk factors for the infection.

MATERIALS AND METHODS

Study areas and animals

A total of 288 blood samples were collected from asymptomatic sheep in June, 2017 in four northern provinces of China, namely Chifeng, Inner Mongolia (n = 76), Jiamusi, Heilongjiang (n = 80), Siping, Jilin (n = 65) and Tangshan, Hebei (n = 67). In each province, the sampling was done in four sheep farms. Details of age, gender and rearing system were recorded. Animals were reared in extensive and intensive systems for meat and were generally kept in herds of 50-100 and more than 100 animals, respectively. In extensive system, sheep are let to roam freely on pastures. Sheep under intensive system, however, are usually caged and fed by the owner.

Approximately 5 ml of blood was obtained from the jugular vein of each sheep, then serum samples were separated after centrifuging at 3,000 × g for 5 min and stored at -20! until tested for antibodies to *T. gondii*.

Serological examination

Antibodies to *T. gondii* were detected using a latex agglutination test (LAT) kit (Toxocheck®-MT, Eiken Chemical, Tokyo, Japan) according to the manufacturer's instructions. In brief, the LAT was carried out in a 96-well U bottomed plate with two-fold dilutions from 1:16 to 1:2,048. The plate was slightly shaken for about 2 min and then incubated at room temperature for least 18 hours without shaking. Test samples at a dilution rate of at least 1:64 that showed a layer of agglutinated latex beads after incubation were considered positive.

Statistical analysis

T. gondii seroprevalence per age groups, management systems, gender and different regions was calculated and compared using the 95% CI of OR (http://www.vassarstats.net). The differences were considered statistically significant if $P < 0.05$.

RESULTS

T. gondii antibody titers by LAT in the 288 sheep are presented in Table 1. Antibody titers in positive samples ranged from 1:64 to 1:1024, the most common titer was 1:64. Overall, 87 samples (30.2%) were positive for anti-*T. gondii* antibodies. The seroprevalence in Hebei (46.3%) was the highest, followed by Heilongjiang (33.8%), Jilin (24.6%) and Inner Mongolia (17.1%). In addition, significant difference was observed between Inner Mongolia and Hebei (Table 2).

The seroprevalence of *T. gondii* was 14.0% in the age group of 0-1 year, 30.9% in 1-2 years and 40.2% in > 2 years. Sheep of less than 1 year were less likely to be seropositive than those of 1-2 and those of > 2 years, and the difference was statistically significant (Table 2).

The seroprevalence in female sheep (32.1%) was slightly higher than in male sheep (26.3%), although the difference was not statistically significant (Table 2). With regards to the rearing system, the seroprevalence in sheep raised intensively (24.2%) was significantly lower than in those raised extensively (37.4%) (Table 2).

Table 1. *T. gondii* antibody titers, by latex agglutination test (cut-off ≥ 64) in 288 sheep sera

Titer	No. of sample (%)	Results by LAT
≤1:32	201 (69.8)	–
1:64	32 (11.1)	+
1:128	17 (5.9)	+
1:256	14 (4.9)	+
1:512	19 (6.6)	+
1:1024	5 (1.7)	+

+ : positive, – : negative.

Table 2. Factors associated with seroprevalence of *T. gondii* infection in 288 sheep in northern China

Characteristics	No. examined	No. positive (%)	Odd ratio (OR) (95% CI)	*P*
Location				
Inner Mongolia – Chifeng	76	13 (17.1)	Reference	
Heilongjiang – Jiamusi	80	27 (33.8)	0.40 (0.19–0.86)	0.014
Jilin – Siping	65	16 (24.6)	0.63 (0.28–1.44)	0.19
Hebei – Tangshan	67	31 (46.3)	0.24 (0.11–0.52)	<0.001
Age (year)				
0–1	57	8 (14.0)	Reference	
1–2	149	46 (30.9)	0.37 (0.16–0.83)	0.009
>2	82	33 (40.2)	0.24 (0.10–0.58)	<0.001
Gender				
Male	95	25 (26.3)	Reference	
Female	193	62 (32.1)	0.75 (0.44–1.30)	0.19
Rearing system				
Extensive	131	49 (37.4)	Reference	
Intensive	157	38 (24.2)	1.87 (1.13–3.11)	0.011
Total	288	87 (30.2)		

Table 3. Reported prevalence rates of *T. gondii* infection in sheep in China

Provinces/cities	Year of sampling	No. tested	Positive (%)	Serologic test[a]	References
Heilongjiang	2008–2010	792	3.0	IHA	Wang *et al.*, 2011
Liaoning	2011	566	4.4	IHA	Yang *et al.*, 2013
Liaoning (Jinzhou)	2012	402	17.9	MAT	Xu *et al.*, 2015
Qinghai (Tibet)	2011	455	5.7	IHA	Wu *et al.*, 2011
Qinghai	2012–2013	600	21.3	ELISA	Liu *et al.*, 2015
Gansu	2013–2014	1732	20.3	MAT	Yin *et al.*, 2015
Yunnan	2012–2013	154	9.7	IHA	Zou *et al.*, 2015
Henan	2015–2016	779	12.7	MAT	Zhang *et al.*, 2016

[a] MAT: modified agglutination test, ELISA: enzyme-linked immunosorbent assay, IHA: indirect hemagglutination.

DISCUSSION

The present study investigated the presence of antibodies against *T. gondii* in sheep from Hebei, Heilongjiang, Jilin and Inner Mongolia, Northern China. The LAT results showed that anti-*T. gondii* antibodies were prevalent in the 4 provinces with significant difference between Inner Mongolia and Hebei. There are two likely reasons for this significant difference. Firstly, the winter season is nearly 5 months and is longer in Inner Mongolia than in Hebei, and the oocysts of *T. gondii* excreted from cat faeces can hardly become infective under low temperatures (Wang *et al.*, 2011). Secondly, the seroprevalence of *T. gondii* in cats is significantly higher (57.3%) in Hebei than in Inner Mongolia (10.3%) (Ding *et al.*, 2017), which may be a major risk factor for the high infection in Hebei, as cats play a significant role in *T. gondii* transmission (Dubey & Schares, 2011).

The overall seroprevalence of *T. gondii* (30.2%) obtained in the present study was far lower than in Bangladesh (69.9%) (Rahman *et al.*, 2014) and India (50%) (Singh *et al.*, 2015), but higher than in some other provinces of China (Table 3). In particular, the prevalence obtained in Heilongjiang province (33.8%) was higher than the 3.1% previously reported in the same province

(Wang *et al*., 2011). The sampling period, sample size and the methods used for selecting the enrolled sheep may have influenced the results obtained in the two reports. In addition, factors including detection methods, age distribution of the samples, management systems, ecological conditions and climates in each investigation may explain the differences between this study and the others. However, all these reports provide strong evidence that *T. gondii* infection is common in sheep in China, and could cause considerable economical loss in sheep.

The seropositivity for *T. gondii* was significantly related to the age of the sheep. This result agrees with previous reports (Ramzan *et al*., 2009; Xu *et al*., 2015), and a similar association has been reported in other species such as cattle (Qiu *et al*., 2012). This suggests the possibility of horizontal transmission in the investigated regions and provides evidence for the increased infection risk for older animals, as they have a longer period to potentially be exposed and infected by *T. gondii*. Animal gender did not affect the seropositivity to *T. gondii* whereas raising system was a significant risk factor. This, in accordance with a previous investigation in Shenyang province of China (Xu *et al*., 2015), indicates that the gender has no significant effect on the prevalence of *T. gondii* in sheep. The effect of rearing system was also reported in Heilongjiang and Liaoning provinces of China (Wang *et al*., 2011; Xu *et al*., 2015). The management practices and environment are strict for the sheep raised intensively. In addition, sheep raised intensively are usually caged. Thus, they have fewer chances to contact or ingest the oocysts of *T. gondii* excreted by infected cats.

In conclusion, the present survey revealed that antibodies to *T. gondii* are widespread in sheep in northern China, which has great significance for public health. In addition, from the results, we can conclude that extensive rearing system is a potential risk factor for *T. gondii* infection in China. The data could provide a foundation for prevention and control of *T. gondii* in sheep in the examined provinces.

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