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Research Note

Seroprevalence and risk factors for *Toxoplasma gondii* in sheep and goats in Jinzhou, Northeastern China

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Abstract. In the present study, serum samples from 402 sheep and 216 goats were collected from 5 counties in Jinzhou from August to October 2012 and antibodies to *Toxoplasma gondii* were detected by modified agglutination test (MAT). Overall, 104 (16.8%) had antibodies to *T. gondii* with antibody titres of 1:25 to 1:800. Seropositive samples were distributed in all the 5 counties and seroprevalences of *T. gondii* varied significantly with flock size, age and rearing system, but not with breed, gender and farm location. The seroprevalences in small farms (18.3%, 95/518, 95% confidence interval [CI], 15.0-21.7%) were statistically higher than that in large farms (9%, 9/100, 95% CI, 3.4-14.6%) ($P < 0.05$), older animals were statistically higher than that in younger animals ($P < 0.01$). The prevalence in extensively and semi-intensively raised samples was statistically higher than that in intensively raised animals ($P < 0.01$). Small flock size and extensive rearing system are the potential risk factors for the prevalence of *Toxoplasma* infection in sheep and goats in Jinzhou. This is the first report of *T. gondii* infection in sheep and goats in Jinzhou, northeastern China, and of an association of seropositivity to *T. gondii* and the risk factors.

Toxoplasma gondii is a protozoan parasite that infects up to a third of the world's population. Infection is mainly acquired by ingestion of food or water that is contaminated with oocysts shed by cats or by eating undercooked or raw meat containing tissue cysts (Dubey & Dubey, 2010; Hide *et al.*, 2009; Montoya & Liesenfeld, 2004). Infection of sheep and goats with *T. gondii* may cause early embryonic death, fetal death and mummification, abortion, stillbirth and neonatal death and thus can be responsible for heavy economic losses (Dubey & Dubey, 2010). The ingestion of undercooked lamb containing tissue cysts of *T. gondii* is considered a significant source of toxoplasmosis in humans (Cook *et al.*, 2000; Dubey, 2000; Gao *et al.*, 2012; Jones *et al.*, 2009). People in Jinzhou have the habit of eating under-cooked 'barbecue', 'kabob' and 'instantly boiled mutton', making increased risk of toxoplasmosis.

Jinzhou is the central city of west part of Liaoning Province, northeastern China, which covers an area of 10,301 square kilometres with a population of 3.07 million. Jinzhou is lying to the north of China's Bohai Sea and the south of Mount Yiwulv, which has a humid continental climate and a relatively large variation in temperature over the course of a year; there are 4 distinct seasons and an annual average rainfall ranging from 540 to

640 mm, heavily concentrated in July and August alone. The geographic and natural climatic conditions are suitable for the development of agriculture, forestry, and livestock production. The livestock industry is an important economic resource in Jinzhou, which has approximately 420,000 sheep and goats. None of the animals had ever been vaccinated against toxoplasmosis; indeed, such vaccination is not practiced in China.

The present study was conducted to investigate the prevalence of *T. gondii* infection and to further explore the potential risk posed to humans in northeastern China.

Serum samples were collected from 618 sheep and goats via a jugular vein in Jinzhou (40°49'–42°08'N, 120°42'–122°36'E), including Heishan, Beizhen, Linghai, Yixian and Taihe from August to October 2012. Whenever possible, data regarding breed, age, gender, location, flock size, rearing system (extensive: daily grazing in favourable weather conditions and returning to fold at night or daily grazing with possibility of shelter in bad weather; intensive: sheep housed day and night) of each animal was collected (Table 1). Blood samples were centrifuged (3,000 rpm) for 5 min and stored at -20°C until use.

The modified agglutination test (MAT) has been evaluated extensively in experimentally and naturally infected sheep and goats, and is demonstrated sensitive and specific for assaying *T. gondii* antibodies in animals (Alvarado-Esquivel, Estrada-Malacon *et al.*, 2013; Chikweto *et al.*, 2011; Ragozo *et al.*, 2008). In the present study, sera were diluted to 1:25, tested for *T. gondii* antibodies (IgG) by MAT as described previously (Dubey & Dubey, 2010). In brief, the harvested parasites were kept in 6% formaldehyde solution at 4°C overnight, and suspended in the alkaline buffer at 20,000 parasites/ml. Two-fold dilutions of sera were performed using the serum diluting buffer, starting with 1:25. Agglutination was done in V-bottom 96-well microtiter plates using a mixture of 50 µl antigen and 50 µl diluted sera. The plates were incubated at 37°C overnight. The test was considered positive when a layer of agglutinated parasites was formed in wells at dilutions of 1:25 or higher; positive and negative controls were included in each test.

Differences in seroprevalence of infected sheep and goats and among associated factors were analyzed using Fisher's exact test in SAS statistical software (Version 9.3; SAS Institute Inc., Cary, NC, USA), 95% confidence intervals (CI) are given. Differences between levels within factors and interactions were considered to be statistically significant and highly significant when $P < 0.05$ and $P < 0.01$, respectively.

Antibodies to *T. gondii* were found in 72 (17.9%) of 402 sheep and 32 (14.8%) of 216 goats in titers of 35 sera with a titer of 25, 24 of 50, 17 of 100, 12 of 200, 9 of 400, and 7 of 800 or higher.

The results of the univariate analysis are shown in Table 1. 16.8% of the 618 tested sheep and goats were seropositive for *T. gondii* by MAT, which was higher than that reported by others (Wang CR *et al.*, 2011; Xu *et al.*, 2014; Zhao *et al.*, 2011). The difference could be associated with management of the sampled animals, ecological conditions, life styles of inhabitants, climates, serological technique used, husbandry practice and the numbers of cats and rodents present. In addition, the feeding conditions and animal welfare are also the risk factors for *T. gondii* infection in sheep and goats (Dubey & Dubey, 2010; Wang CR *et al.*, 2011).

The logistic regression showed that all the factors (breed, sex, age, location, flock size and rearing systerm) reported in the present study affected prevalence of infection (Table 1). The seroprevalence in sheep (17.9%, 72/402, 95% CI, 14.2-21.7%) was higher than that in goats (14.8%, 32/216, 95% CI, 10.1-19.6%), but the difference was not statistically significant ($P > 0.05$). Although the prevalence of *T. gondii* infection in sheep and goats across the world is variable, the literature generally indicates that the prevalence of infection amongst sheep is higher than that in goats (Kamani, Mani & Egwu, 2010; Lopes *et al.*, 2013). The seroprevalence in female animals (18.3%, 81/443, 95% CI, 14.7-21.9%) was not found to be

Table 1. General characteristics of the 618 sheep and goats studied and seroprevalence of *T. gondii* infection[a] in Jinzhou, northeastern China

Characteristics	No. examined	No. positive	Prevalence, % (95% CI)	P
Breed				0.323
Sheep	402	72	17.9 (14.2,21.7)	
Goat	216	32	14.8 (10.1,19.6)	
Age(year)				0.001
yr≤1	86	4	4.7(1.3,11.4)	
1<yr≤3	307	50	16.3 (12.2,20.4)	
3<yr	225	50	22.2(16.8,27.7)	
Gender				0.124
Male	175	23	13.1 (8.1,18.2)	
Female	443	81	18.3 (14.7,21.9)	
Location (County)				0.591
Heishan	156	31	19.9 (13.6,26.1)	
Beizhen	125	23	18.4 (11.6,25.2)	
Yixian	132	22	16.7 (10.3,23.0)	
Linghai	108	14	13.0 (6.7,19.3)	
Taihe	97	14	14.4 (7.4,21.4)	
Flock size				0.022
Large(n≥100)	100	9	9.0(3.4,14.6)	
Small(n<100)	518	95	18.3(15.0,21.7)	
Rearing system				0.005
Extensive	58	15	25.9(14.6,37.1)	
SIR[b]	400	74	18.5(14.7,22.3)	
IR[c]	160	15	9.4(4.9,13.9)	
Total	618	104	16.8 (13.9,19.8)	

[a]The difference was considered significant when P value less than 0.05; CI, Confidence interval; [b]SIR, semi-intensively raised, [c]IR, intensively raised.

significantly higher ($P > 0.05$) than that in males (13.1%, 23/175, 95% CI, 8.1–18.2%). This gender-related tendency of prevalence had been reported previously (Wang CR *et al.*, 2011; Xu *et al.*, 2014), however, a study in Nigeria (Kamani *et al.*, 2010) did not find an association between seroprevalences and genders.

In the present study, seroprevalence in sheep and goats increased progressively with age, ranging from 4.7%–22.2%, with the highest of 22.2% in samples which were >3 year old, the seroprevalences were statistically significantly higher than that in younger groups (≤1-year-old) ($P < 0.01$). These results are similar to those of previous investigations (Alvarado-Esquivel, Silva-Aguilar, Villena, & Dubey, 2013; Ramzan *et al.*, 2009), suggesting the possibility of horizontal transmission in the investigated herds.

In the present survey, the seroprevalence in small farms (18.3%, 95/518, 95% CI, 15.0–21.7%) was statistically higher ($P < 0.05$) than that in large farms (9%, 9/100, 95% CI, 3.4–14.6%). In Jinzhou, sheep and goats feeding is predominated by small-scale rearing by farmer households, compared with other

district. Thus, sheep have more chance to ingest the oocysts of *T. gondii* excreted by infected cats in poor breeding conditions of small farms. In addition, sheep and goats are raised extensively in small farms or semi-intensively by individual families in the study region. And the prevalence in intensively raised sheep and goats was statistically lower ($P < 0.01$) than that in extensively and semi-intensively raised samples (Table 1). Our findings are similar to those of previous reported (Ragozo *et al.*, 2008; Wang CR *et al.*, 2011). The main reason for such a difference may be that, compared with extensively or semi-intensively raised animals, intensively raised sheep and goats are caged and thus have less chance to ingest the oocysts of *T. gondii* excreted by infected cats.

The prevalence of *T. gondii* infection varied from 14.4% to 19.9% among different regions in Jinzhou district, and the differences among seroprevence of *T. gondii* in different regions are shown in Table 1. Higher prevalence was found in Heishan (19.9%, 31/156, 95% CI, 13.6–26.1%), compared with other regions. Previous studies have shown that *T. gondii* infection in free-range chickens was a good indicator of the environmental contamination with oocysts because chickens became infected mainly by feeding from ground, feed, or soil contaminated with oocysts (Beltrame *et al.*, 2012; Dubey, Lenhart, *et al.*, 2005; Dubey, Rajapakse, Ekanayake, Sreekumar & Lehmann, 2005). Although the data collected in our study did not include the positive association between the presence of free-range chickens and *T. gondii* infection in sheep, previous study had shown that a high rate (20%) of *T. gondii* infection was found in free-range chickens from Heishan (Xu *et al.*, 2012), which may contribute to the higher prevalence of *T. gondii* infection in sheep and goats from the same region.

Based on the results obtained in this study, it can be concluded that small flock size and extensive rearing system are the potential risk factors for *Toxoplasma* infection in sheep and goats in Jinzhou, northeastern China. Control and prophylactic measures should be adopted to improve the rearing system and the implementation of health promoting programs in a joint effort between farmers, farmers' associations and veterinarians to inform about the means of transmission of the infection and for a better understanding of toxoplasmosis.

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