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Short Communication

Seroprevalence of *Toxoplasma gondii* Infection in Tibetan Pigs in Nyingchi, Tibet, China

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JKL, RRL and KL contributed in the plan of the study, while JKL, RRL, KL, XQW, HQL, GQ, HZ and YFL preformed the experiments. JRL, RRL and KL analyzed the data and wrote the manuscript.

Key words
***Toxoplasma gondii*, Tibetan pigs, Enzyme-linked immunosorbent assay, Indirect hemagglutination test, Seroprevalence.**

ABSTRACT

The survey was to investigate the seroprevalence of *T. gondii* in Tibetan pigs in Tibet, China. A total 454 serum samples were collected from Tibetan pigs in Nyingchi in 2014 and 2015. Each sample was assayed for *T. gondii* antibodies by a commercial enzyme-linked immunosorbent assay kits and an indirect hemagglutination test. The results showed that seroprevalence in Tibetan pigs were 25.6% by ELISA. On gender basis, the prevalence was found 25.1% in males and 27.8% in females. By the method of IHA, the seroprevalence in Tibetan pigs were 21.6%, and the prevalence was found 23.1% and 21.4% in males and females, respectively. The results indicates that the Tibetan gigs were exposed to *T. gondii* in Tibet area, which should arise a public concern of the threat to human and other animals in this unique region of the world.

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan parasite which has a worldwide distribution infects both humans and animals. The two principal transmission routes of this organism are the ingestion of oocysts from cat feces contaminated food or water; and the ingestion of viable cysts found in raw or under cooked meat (Dubey, 2010). *T. gondii* destroys the reproductivity of pigs, including abortion, stillbirth and fetal mummification in females first infected during pregnancy (Tsutsui *et al*., 2003). Generally, the infestation in humans is asymptomatic (Dubey, 2010); however, previous study had reported that pregnant women infected *T. gondii* may cause severe disease with vertical transmission to the fetus, including abortion, encephalitis, mental retardation, and blindness (Cook *et al*., 2000). More importantly, to immunocompromised patients including AIDS patients and organ transplant recipients, *T. gondii* infection can cause serious or fatal hazard (Montoya and Liesenfeld, 2004).

Tibetan pig is a local animal mainly distributed in the Southeast of Tibetan area fed by the free range. Mainly with the character of disease resistance, this animal can easily be bred with high quality lean carcass, and is an important agricultural product for Tibetans (Wu *et al*., 2012a; Zhang *et al*., 2014). So, at this high altitude, any parasite infection in pigs may cause significant economical loss in pig production and have a potential threat to infect native farmers. However, to date, limited information for *T. gondii* infection in Tibetan pigs is available in Tibet, China. The present study herein was designed to survey the seroprevalence and identify genotypes of *T. gondii* infection in Tibetan pigs in this area.

Materials and methods

A total 454 serum samples from Tibetan pigs with an age of 1.5-2 years were collected in a slaughter house in Nyingchi prefecture between December to February 2014-15, and from different counties of Nyingchi prefecture (Table I). After collection, each of the samples were centrifuged at 4000 × g for 10 min, and serum was separated and stored at -20°C till further analysis. In addition, 31 tissue samples (17 livers and 14 lungs) coming from 19 Tibetan pigs were collected and stored at -20°C.

All serum samples were tested for immunoglobulin G antibodies to *T. gondii* using a commercial enzyme-linked

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immunosorbent assay kits and indirect agglutination test (ELISA, Lanzhou Veterinary Research Institute of Chinese Academy of Agricultural Sciences) according to the manufacturer's instructions. For ELISA, the test was considered positive when the OD (490nm) value of the sample was twice of OD (490nm) value of negative control. Positive and negative controls were included in each test. For IAT the test was considered positive when a layer of agglutinated erythrocytes was formed in wells using serum dilutions of 1:64 or higher, and positive and negative controls were included in each test (Li *et al*., 2014).

Statistical analysis was performed by chi-square test with SPSS (Statistical Analysis System, Version 17.0.). The differences were considered statistically significant when $P<0.05$.

Table I.- Seroprevalence of *T. gondii* infection in Tibetan Pigs and different gender from different regions of Nyingchi, Tibet by ELISA and IHA, respectively.

County	Samples	ELISA	IHA
		Positive serum/ Seroprevalence (%)	Positive serum/ Seroprevalence (%)
Tibetan pigs			
Nyingchi	133	29/21.8%	23/17.3%
Gongbo'gvamda	128	38/29.7%	35/27.3%
Mainling	193	49/25.4%	40/20.7%
Total	454	116/25.6%	98/21.6%
Gender			
Male	251	63/25.1%	58/23.1%
Female	187	52/27.8%	40/21.4%
Unknown	16	1/6.3%	0/0

Results

A total 116 out of 454 tested samples were found positive for antibodies to *T. gondii* with the OD (490nm) value twice of negative control. The regional seroprevalence of *T. gondii* was 100% (3/3). The prevalence in each region in Tibet ranged from 21.8% to 29.7%, and differences among the regions were found statistically insignificant (Table I). The prevalence in male pigs and female pigs were observed 25.1% and 27.8%, respectively and the differences between genders were also found statistically insignificant (Table I).

A total 98 out of 454 serum samples were observed positive for antibodies to *T. gondii* with titers of 1:64 or higher. The regional seroprevalence of *T. gondii* was 100% (3/3). The prevalence of *T. gondii* infection in each region in Tibet ranged from 17.3% to 27.3%, and differences among the regions were statistically insignificant (Table I). The prevalence in male pigs and female pigs were 23.1% and 21.4%, respectively. The differences between genders were also statistically insignificant (Table I).

One serum sample was tested as negative by IAT which was found positive through the test of ELISA. The seroprevalence tested by IAT were lower than tested by ELISA, and there were no significant difference between the two techniques ($p \geq 0.05$).

Discussion

Since the discovery of complete life cycle of *T. gondii* in 1970, a large number of wild and domestic animals have been identified as intermediate hosts (Tenter, 2009). Previous study have shown that the prevalence of *T. gondii* infection in pigs in Jiangxi Province, Southeastern China was 22.9% (Jiang *et al*., 2014). Zhou *et al*. (2010) and Wu *et al*. (2012a) have reported that the prevalence of the prevalence of *T. gondii* infection in pigs in Guangdong and Chongqing, China was 27.0% and 30.6%, respectively. In Xingjian, the neighboring province of Tibet, the prevalence of *T. gondii* infection in pigs is 47.2% (Lu *et al*., 2014). In the present study, the research was conducted in a wide area covering most of the Nyingchi prefecture in Tibet. Our data suggested that the prevalence of *T. gondii* infection in Tibetan Pigs in Tibet were found 25.6% by ELISA and 21.6% by IHA. The variable 'gender' did not appear to be important for infection by *T. gondii* ($P\geq0.05$). This result indicated that both the genders were equally exposed to the risk of infection. Azevedo *et al*. (2010) and Lopes *et al*. (2013) also found no gender-related differences in their research. The present study and a previous one (Wu *et al*., 2012b) demonstrate the high prevalence of *T. gondii* infection in Tibetan Pigs. Although the prevalence of *T. gondii* in 2015 is between the data of previous reports; however the seroprevalnce depicted an increasing trend of this parasite in Tibetan pigs from 2012 to 2015.

Tibetan pigs are usually raised in an outdoor environment and are more likely to ingest food or water contaminated with *T. gondii* oocysts excreted by infected felids (Wu *et al*., 2012b). This may be the one of the reasons of high prevalence of this protozoan on the Tibet plateau as many wild animals like pikas, yaks were reported of infecting *T. gondii* (Zhang *et al*., 2013; Li *et al*., 2014). This prodigious breeder which serves as the favorite food of carnivores living in the area is considered to be a reservoir of environmental spread of *T. gondii* on the plateau (Zhang *et al*., 2013). Domestic and wild cats are its definitive hosts, and the domestic cat (*Felis catus domesticus*) is the major host (Feitosa *et al*., 2014). Tibetan especially like the cats and it is a very common thing that almost every herdsman's family own cats which

may also contribute to the high growth rate of *T. gondii* infection and there are a potential threats to the health of local residents. The mild climatic changes and increasing trade in recent years might also have made the contribution to the high growth rate of *T. gondii* infection in the region (Li *et al*., 2014).

Consumption of under cooked or raw meat or meat products is also an important route of human infection with *T. gondii* (Jiang *et al*., 2014; Ahmad and Tasawar, 2016). Moreover, people dealing with such type of meat by poor hygienic measures may also become infected during evisceration and handling of the carcass (Tenter, 2009). In China, pork is the most common meat, accounting for 65% of the meat consumption. However, before it is sold and consumed, pork is not required to be inspected for *T. gondii* contamination by law (Jiang *et al*., 2014). As the high prevalence of *T. gondii* infection in Tibetan pigs presented herein indicated the rising possibility of this protozoan to infect other animals and ultimately to humans on the Tibetan Plateau. Hence, it is necessary to improve management of pig farming and pig *T. gondii* infection detection to reduce *T. gondii* transmission for public health concern.

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Conflict of interest declaration

We declare no conflict of interest.

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