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First Report of *Toxoplasma gondii* Prevalence in Tibetan Pigs in Tibet, China

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Abstract

Toxoplasma gondii infection is widely prevalent in humans and animals, including pigs throughout the world. In this study, the seroprevalence of *T. gondii* infection in Tibetan pigs in China was investigated for the first time. A total of 427 serum samples were collected from Tibetan pigs in Nyingchi prefecture, Tibet, between April and December 2010, and were assayed for antibodies to *T. gondii* using the modified agglutination test (MAT). Ninety-seven (22.72%) pigs were found to be positive with MAT titers of 1:25 or higher. Slaughter pigs had the highest seroprevalence, compared with seroprevalence in fattening pigs, growing pigs, or piglets, although the difference was not statistically significant ($p \geq 0.05$). The results of the present survey indicate that *T. gondii* is highly prevalent in Tibetan pigs in Tibet, which poses a significant public health concern in this unique region of the world.

Key Words: Modified agglutination test (MAT)—Survey–Tibet–Tibetan pigs–*Toxoplasma gondii*–Toxoplasmosis.

Introduction

Toxoplasma gondii is an important intracellular protozoan parasite of zoonotic significance, affecting humans and animals worldwide (Tenter et al. 2000; Montoya and Liesenfeld 2004; Dubey 2009, 2010; Jittapalapong et al. 2011; Vujanić et al. 2011; Zhou et al. 2011; Elmore et al. 2012). In the People's Republic of China, it is estimated that approximately 7.9% of the population has been exposed to *T. gondii*, with an increasing incidence in recent years (Zhou et al. 2011). Generally, *T. gondii* infection in humans is asymptomatic (Dubey 2010), but primary infection in pregnant women may cause severe disease with vertical transmission to the fetus, leading to abortion, encephalitis, mental retardation, and blindness (Cook et al. 2000). More importantly, *T. gondii* infection can be fatal in immunocompromised patients, such as AIDS patients and organ transplant recipients (Montoya and Liesenfeld 2004).

The parasite can also severely threaten the health of pigs, and clinical signs in pigs vary from symptomless infections to death. Sow reproductive performance may also be affected, leading to heavy economic losses to the swine industry (Dubey 2009). Pig meat is an important foodstuff worldwide, and humans can acquire infections with *T. gondii* by ingesting undercooked pig meat containing tissue cysts. Surveys of seroprevalence of *T. gondii* infection in pigs have been reported throughout the world (Dubey 2009, 2010; Garcia-Bocanegra et al. 2010; Holec-Gasior et al. 2010), and there have also been surveys of *T. gondii* infection in pigs in several provinces of China in recent years (Suo et al. 2006; Zou et al. 2009; Huang et al. 2010; Zhou et al. 2010).

Tibetan pigs are mainly distributed in the Qinghai-Tibetan Plateau, and usually feed on wild plants outdoors. The typical characteristics of the Tibetan pig is its powerful musculature, and the fact that its entire body is covered by hard black hair (Li and Luo 1993; Zheng 1998). Tibetan pig meat is a sought-after delicacy, which is high in protein, with a tender texture, and rich in amino acids, and thus is an important source of protein for Tibetans. However, little is known about the prevalence of *T. gondii* in Tibetan pigs in Tibet. In view of this

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background, the objective of this survey was to estimate the seroprevalence of *T. gondii* infection in Tibetan pigs.

Materials and Methods

The study site

Nyingchi prefecture is situated in southeast Tibet, between eastern longitude 94°29′ and northern latitude 29°35′, sharing borders with Yunnan Province and Qamdo prefecture in the east and northeast, Naqu prefecture in the north, Lhasa city in the west, Shannan prefecture in the southwest, and India and Myanmar in the south. The average height of this area is 3100 meters above sea level, covering about 117,000 km^2, with a population of more than 140,000. It has an annual rainfall of 650 mm, annual average temperature of 8.7°C, yearly sunlight exposure of 2022.2 h, and a frost-free season of about 180 days.

Naturally-infected Tibetan pigs

A total of 427 blood samples were collected from the two counties of Nyingchi prefecture between April and December 2010 (Table 1). These pigs were raised by local Tibetans, and were usually left outdoors and free-ranging to forage for food. The Tibetan pigs were categorized according to age as follows: piglet <3 months, growing pig ≥ 3 months and <24 months, fattening pig ≥ 24 months and <36 months, slaughter pig ≥ 36 months, breeding sow (adult female) ≥ 24 months, and breeding boar (adult male) ≥ 24 months. The blood samples were left at 37°C for 2 h and centrifuged at 3000 *g* for 5 min to obtain serum. These sera were stored at -20°C until further analysis.

Serological examination

Serum samples from Tibetan pigs were diluted twofold from 1:25 to 1:1600 and examined for *T. gondii* antibodies using the modified agglutination test (MAT) as described previously (Dubey and Desmonts 1987; Wu et al. 2011a, 2011b). In this study, sera with MAT titers of 1:25 or higher were considered positive, because *T. gondii* can be isolated from naturally-infected pigs with antibody titers as low as 1:25 by MAT (Dubey et al. 1995a, Dubey 1997). Positive and negative control sera were incorporated into each test. The positive control sera were collected from pigs experimentally infected with *T. gondii*. The negative control sera were collected from pigs without *T. gondii* infection (collected before experimental infection). Those sera with questionable results were re-tested.

Table 1. Seroprevalence of *Toxoplasma gondii* Infection in Tibetan Pigs in Tibet, China, as Determined by the Modified Agglutination Test

Type of pig	*No. examined*	*No. positive*	*Prevalence (%)*
Breeding boar	4	0	0
Breeding sow	5	0	0
Slaughter pig	110	29	26.36
Fattening pig	106	25	23.58
Growing pig	131	28	21.37
Piglet	71	15	21.13
Total	427	97	22.72

Statistical analysis

Differences in *T. gondii* seroprevalence between different groups of Tibetan pigs were analyzed using a chi-square test by SPSS for Windows (release 18.0 standard version; SPSS Inc., Chicago, IL). Differences were considered statistically significant when $p<0.05$.

Results and Discussion

Table 1 shows the seroprevalence of *T. gondii* in 427 Tibetan pigs, with an overall prevalence of 22.72% (97/427). The distribution of seropositives was as follows: 26.36% (29/110) in the slaughter pigs, 23.58% (25/106) in the fattening pigs, 21.37% (28/131) in the growing pigs, and 21.13% (15/71) in the piglets. No seropositive pigs were detected among breeding boars and sows, possibly due to the limited serum samples. Antibodies ($\geq$1:25) were found in 97 of 427 (22.72%) Tibetan pigs with MAT titers of 1:25 in 18, 1:50 in 10, 1:100 in 1, 1:200 in 28, 1:400 in 13, 1:800 in 9, and 1:1600 or higher in 18.

The overall *T. gondii* seroprevalence in Tibetan pigs in Nyingchi prefecture, Tibet, was lower than that reported in pigs in southern China's subtropical Hainan and Guangdong Provinces (Suo et al. 2006; Zhou et al. 2010). This is possibly related to different climate conditions in these areas. The average annual temperatures in Hainan, Guangdong, and Nyingchi are 23.8°C, 22.3°C, and 8.7°C, respectively, and the climate in Hainan and Guangdong Provinces is warmer and more humid than in Nyingchi prefecture. *T. gondii* oocysts are more prone to become infective and survive longer in warmer and more humid environments (Dubey 2010). Slaughter pigs had the highest *T. gondii* seroprevalence, compared with the seroprevalence in the fattening pigs, growing pigs, and piglets, possibly due to difference in their ages, although the difference was not statistically significant ($p \geq 0.05$, data not shown). Although the average annual temperature in Nyingchi prefecture is low, Tibetan pigs are usually raised in an outdoor environment and so have more potential contacts with felids and rodents, which are more common than in some other parts of China; they also are more likely to ingest food or water contaminated with *T. gondii* oocysts excreted by infected felids.

Previous investigations have shown that dietary habits play an important role in *T. gondii* infection in China (Zhou et al. 2011). *T. gondii* seroprevalence was higher in some ethnic groups who eat raw or undercooked meat (Zhou et al. 2011). Although the *T. gondii* seroprevalance in Tibetans in Nyingchi prefecture is not yet known, indigenous people in this area like to eat roast pork derived from Tibetan pigs, and this may result in a high *T. gondii* prevalence in Tibetans in this area.

In this study, we used the MAT for the detection of *T. gondii* seroprevalence in Tibetan pigs because of its high sensitivity and specificity for the diagnosis of latent toxoplasmosis compared to other serological tests (Dubey et al. 1995b). Previous studies have shown that the MAT is a specific technique for measuring *T. gondii* antibodies in pigs, and no cross-reaction was found when using the MAT for testing serum samples of *T. gondii*-negative pigs infected with other parasites and viruses (Dubey 1997, 2009, 2010).

In conclusion, the results of the present survey demonstrate that *T. gondii* is prevalent in Tibetan pigs, indicating the widespread contamination of the environment by *T. gondii* oocysts in Nyingchi, Tibet. This poses a significant public health risk in Tibet because *T. gondii* is an important zoonotic

parasite, and the meat of Tibetan pigs is an important part of the Tibetan diet. Therefore, measures should be taken to prevent cat feces from contaminating the environment in Tibet. The results also provide foundational data for executing integrated strategies and measures to control toxoplasmosis in humans and animals in Tibet.

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Author Disclosure Statement

No competing financial interests exist.

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