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Seroprevalence of *Toxoplasma gondii* infection in pigs in Jilin Province, Northeastern China

Xu, P.[1,2†], Cai, Y.N.[1†], Leng, X.[3], Wang, J.[1,2], Ma, W.[1,2], Mu, G.D.[4], Jiang, J.[1], Liu, X.Y.[1], Wang, Z.D.[5], Zhao, Q.[1*] and Yang, G.L.[1*]

[1]College of Animal Science and Technology, Jilin Agricultural University, Changchun, Jilin Province 130118, P.R. China

[2]College of Animal Husbandry and Veterinary Medicine, Liaoning Medical University, Jinzhou, Liaoning Province 121001, P.R. China

[3]State Key Laboratory for Molecular Biology, Institute of Special Wild Economic Animal and Plant Science, Chinese Academy of Agricultural Science, Changchun, Jilin Province 130122, P.R. China

[4]Jilin Center for Animal Disease Control and Prevention, 4510 Xi'an Road, Changchun, Jilin Province 130062, P.R. China

[5]Institute of Military Veterinary, Academy of Military Medical Sciences, Key Laboratory of Jilin Province for Zoonosis Prevention and Control, 666 Liuying Xilu, Changchun, Jilin Province 130122, P.R. China

[†]These authors equally contributed as the first author

[*]Corresponding author email: zhaoquan0825@163.com, dyygl@163.com

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Abstract. In the present study, a total of 1,235 porcine serum samples were collected from 9 counties in Jilin Province (40°52'~46°18'N,121°38'~131°19'E), northeastern China from August to October 2013, and the seroprevalence of *Toxoplasma gondii* infection was tested by indirect haemagglutination assay (IHA). The results showed that antibodies to *T. gondii* were found in 19.1% (95% confidence interval [CI], 16.9% to 21.3%), with higher seroprevalence in the breeding boars (28.6%, 95% CI, 20.0% to 37.2%), and breeding sows (32.0%, 95% CI, 25.2% to 38.9%). No significant difference was found among the slaughter pigs, fattening pigs and the piglets. These results indicated that infection with *T. gondii* in pigs is widespread in Jilin province, and is of public health concern.

INTRODUCTION

Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, is an important food-borne parasitic zoonosis worldwide. Food-producing animals pose a risk for transmission of the disease to humans, mainly by ingesting tissue cysts from undercooked or raw meat (Dubey, 2009). Since pork is the most commonly consumed meat by Chinese people, it is important to investigate the prevalence of *T. gondii* infection in pigs in China. Jilin Province has a human population of 27 millions, and is one of the largest pork producers in China, accounting for 70% of the livestock industry's total income (Zhou *et al*., 2010; Tao *et al*., 2011). The objective of the present study was to investigate seroprevalence of *T. gondii* infection in pigs in Jilin province, Northeastern China.

MATERIALS AND METHODS

Study areas

Jilin Province is about 600 km from north to south and about 750 km from east to west. It is boarded by Liaoning Province on the south, Inner Mongolia Autonomous Region on the west, Heilongjiang Province on the north and Russian Federation on the east, and its southeast is on opposite side of Democratic People's Republic of Korea. Jilin is an inland and border province near the sea. The province has a distinct temperate continental monsoon climate with a clear-cut change of

four seasons. The yearly average temperature of most part of the province is 3-5ºC. The frost period begins in the last 10 days of September and lasts until the end of April or early May.

Sampling and sera preparation

Blood samples were collected from 1,235 pigs from slaughter houses or farms in Jilin Province, including Changchun (43º05' –45º15' N, 124º18' –127º02' E), Jilin (42º31' – 44º40' N, 125º40' –127º56' E), Siping (42º56' – 44º05' N, 123º25' –125º05' E), Liaoyuan (42º17' – 43º13' N, 124º51' – 125º49' E), Tonghua (40º52' –43º3' N, 125º10' –126º44' E), Baishan (44º13' – 46º18' N, 121º38' – 124º22' E), Songyuan (43º59' – 45º32' N, 123º6' – 126º11' E), Baicheng (44º13' – 46º18' N, 121º0' – 124º22' E), Yanbian (42º50' – 43º23' N, 129º1' – 129º48' E) from August to October, 2013. Sera were separated by centrifugation at 1,500x*g* for 5 min and stored at -20ºC until use.

Serological examination

Antibodies against *T. gondii* (IgG) were detected in sera by an indirect hemagglutination antibody (IHA) test using a commercially marketed kit (Veterinary Research Institute, Jiangsu Academy of Agricultural Sciences, Nanjing, China) according to the manufacturer's instructions (Zou *et al*., 2009). In brief, sera were added to 96 well V bottomed polystyrene plates, and diluted in a four-fold series from 1:4 to 1:2048. The plates were shaken for 2 min and then incubated at 37ºC for 2 h without shaking. The test was considered positive when a layer of agglutinated erythrocytes was formed in wells at dilutions of 1:64 or higher, and positive and negative controls were included in each test. The cutoff value of 1:64 was used according to the national standard (GB/T 18448.2-2008) of China for detection of *T. gondii* antibodies in humans and animals. IHA kit has been extensively used for detecting specific antibodies to *T. gondii* in pigs, sheep, and other mammals in China for many years. IHA shows a sensitivity of about 89.8%, but the specificity can reach 96.6% for detecting *Toxoplasma* IgG antibody (Yang *et al*., 2014).

Statistical analysis

The difference of *T. gondii* prevalence in different groups was analysized by Fisher's exact test using SAS statistical software (Version 9.3; SAS Institute Inc., Cary, NC, USA), and *P* values <0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Antibodies to *T. gondii* were found in 236 (19.1%) of 1235 pigs in titers of 103 sera with a titer of 64, 73 of 128, 38 of 256, 17 of 512, and 5 of 1024, respectively. The results of the univariate analysis are shown in Table 1. Higher seroprevalence ($P < 0.05$) was found in the breeding boars and breeding sows compared to slaughter pigs, fattening pigs and piglets. The results were in accordance with previous studies (Garcia-Bocanegra *et al*., 2010). The possible reason could be that the old animals were more frequently exposed to *T. gondii*, and that the environment may play an important role as a source of infection. No significant difference ($P>0.05$) was found between the breeding boars and breeding sows, or among the slaughter pigs, fattening pigs and piglets, indicating that high level of vertical transmission in *T. gondii* in pigs may occur in Jilin Province, but it still needs more information to fully convince this deduction. In fact, previous studies have shown that congenital transmission of this parasite plays an important role in ovine and murine toxoplasmosis (Innes *et al*., 2009; Garcia-Bocanegra *et al*., 2010; Thomasson *et al*., 2011; Edwards & Dubey, 2013).

The prevalence of *T. gondii* infection varied from 8.2% to 38.8% among different regions in Jilin Province (Table 1). The differences among seroprevalence of *T. gondii* in different regions are shown in Table 2. Higher prevalence ($P < 0.05$) was found in Baishan and Baicheng, compared with the other regions. Previous studies have shown that the presence of cats and rodents on the pig farms was the main risk factor for *T. gondii* infection in pigs, and cats are essential for the maintenance of *T. gondii* infection in pig farms through contamination

Table 1. Seropevalence of *Toxoplasma gondii* infection in pigs by IHA in Jilin Province, northeastern China[a]

Type and origin of pigs	No. sample	No. positive	Prevalence, % (95% CI)
Types of pig			
Breeding boar	105	30	28.6 (20.0-37.2)
Breeding sow	178	57	32.0 (25.2-38.9)
Slaughter pig	225	33	14.7 (10.0-19.3)
Fattening pig	597	102	17.1 (14.1-20.1)
Piglet	130	14	10.8 (5.4-16.1)
Origin of pig			
Baicheng	80	31	38.8 (28.1-49.4)
Baishan	110	36	32.7 (24.0-41.5)
Tonghua	90	20	22.2 (20.3-38.3)
Songyuan	120	26	21.7 (14.3-29.0)
Siping	120	22	18.3 (11.4-25.3)
Yanbian	195	34	17.4 (12.1-22.8)
Changchun	185	27	14.6 (9.5-19.7)
Jilin	250	33	13.2 (9.0-17.4)
Liaoyuan	85	7	8.2 (2.4-14.1)
Total	1235	236	19.1 (16.9-21.3)

[a]Seroprevelances of *T. gondii* infection in pigs from different types were analyzed using Fisher's exact test, and higher seroprevalence was found in the breeding boars and breeding sows than slaughter pigs, fattening pigs and piglets ($P<0.05$).

Table 2. *P* values of comparison of seroprevalence of *Toxoplasma gondii* infection in pigs from different locations by Fisher's exact test

Location	Jilin (13.2%)	Siping (18.3%)	Liaoyuan (8.2%)	Tonghua (22.2%)	Baishan (32.7%)	Songyuan (21.7%)	Baicheng (38.8%)	Yanbian (17.4%)
Changchun (14.6%)	0.67599	0.42617	0.16958	0.00456[a]	0.0003[a]	0.12332	0.00004[a]	0.48644
Jilin (13.2%)		0.21289	0.25149	0.00094[a]	0.00003[a]	0.04803[a]	0.00000[a]	0.23096
Siping (18.3%)			0.04401[a]	0.07668	0.01490[a]	0.62861	0.00182[a]	0.87987
Liaoyuan (8.2%)				0.00033[a]	0.00004[a]	0.01168[a]	0.00000[a]	0.06456
Tonghua (22.2%)					0.65437	0.21298	0.20468	0.02396[a]
Baishan (32.7%)						0.07441	0.44290	0.00289[a]
Songyuan (21.7%)							0.01064[a]	0.37732
Baicheng (38.8%)								0.00028[a]

[a] The difference was considered significant when *P* value less than 0.05.

of feed and/or water by oocyst (Dubey *et al.*, 1991). In the present study, we did not examine cats or rodents on pig farms. Previous study has shown that a high rate (50.4%) of *T. gondii* infection was found in rodent, *Microtus fortis* from Baicheng (Zhang *et al.*, 2013), which may contribute to the higher prevalence of *T. gondii* infection in pigs from the same regions. In addition, the feeding conditions, animal welfare, and ecological conditions are also the risk factors for *T. gondii* infection in pigs. In Jilin Province, swine feeding is predominated by small-scale breeding by farmer households,

compared with other province. Thus, pigs have more chance to ingest the oocysts of *T. gondii* excreted by infected cats in poor breeding conditions of small farms.

The present survey showed that the overall seropositivity for *T. gondii* infection in pigs was 19.1% in Jilin Province, which was lower than the values of 30.6% in pigs in a study conducted in Chongqing (Wu *et al.*, 2012), 22.9% in Jiangxi (Jiang *et al.*, 2014), 33.3% in Hunan (Xu *et al.*, 2014) and 53.4% in Zhejiang (Yu *et al.*, 2011), but higher than that observed in Heilongjiang (4.6%) (Chang *et al.*, 2013), and similar to that of Yunnan (17.0%) (Zou *et al.*, 2009). *Toxoplasma gondii* seroprevalence in pigs may be affected by ecological and geographical factors. It is known that the Jilin province has a northerly continental monsoon climate, with long cold winters and short warm summers. Average January temperatures range from -20 to -14ºC. Dry and cold circumstances may be unfavorable for the survival of *T. gondii* oocysts. The seroprevalence in Jilin and Yunnan are quite similar, which may be due to small-scale raising, different socio-economic conditions, types of pigs surveyed and the management of pigs.

Although the difference was not statistically significant, the older pigs had the higher prevalence of *T. gondii* infection, compared with younger animals. The probable reason is in accordance with those previously reported (Zhou *et al.*, 2010; Garcia-Bocanegra *et al.*, 2010) as an indication of increased contact with age and to lifelong persistence of antibodies. Moreover, the higher seroprevalence in adult pigs could also be related to the immunosuppression caused by the combination of pregnancy-associated stress and hormonal changes (Zou *et al.*, 2009).

In summary, the present study showed that *T. gondii* infection is relatively high in pigs in Jilin province, northeastern China, which may represent a potential source for human infection with *T. gondii*. Therefore, enhanced and integrated strategies must be implemented to prevent and control *T. gondii* infection in pigs.

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