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Seroprevalence and the Risk Factor of *Toxoplasma gondii* Infection to Slaughter Pigs in Chongqing, China

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Abstract

Toxoplasma gondii can infect all mammals, including humans, and can cause serious public health problems and economic losses. Pork is considered an important source of infection for humans, and seroepidemiological surveys are used to assess the level of infection in pig herds. To understand the current seroprevalence and potential risk factors of *T. gondii* in pigs in Chongqing, a total of 1221 serum samples collected from seven slaughterhouses in five districts from 2015 to 2019 were analyzed for antibodies against the protozoan by enzyme-linked immunosorbent assay. The overall seropositive rate for *T. gondii* antibodies in Chongqing was 11.1%. The rate varied among the different districts (9.8–15.2%), slaughterhouses (8.0–18.4%), seasons (8.1–14.6%), and years (6.3–14.7%). These results suggest that the season and year were potential risk factors for *T. gondii* infection in pigs of Chongqing. The study provides prevalence and risk factor data that may help manage livestock and human infections in downstream areas. The Clinical Trial Registration number was SV-20150605-01.

Keywords: *Toxoplasma gondii*, pig, ELISA, seroprevalence, risk factor, Chongqing

Introduction

TOXOPLASMA GONDII IS an obligate intracellular parasitic protozoan that can invade most nucleated cells and cause serious harm to humans and livestock health. It has a high infection rate and low disease incidence. Cats are the only terminal host, while other mammals, birds, and humans are intermediate hosts (Dubey et al. 2009, 2010).

About one-third of the world's population is infected with *T. gondii* (Djokic et al. 2016), but most infections are recessive. However, immunodeficiency groups (including cancer patients and AIDS patients) can be more seriously affected by the toxoplasmosis. In humans, *T. gondii* infection during pregnancy can lead to fetal diseases, such as abortion, stillbirth, mummification, mental retardation, and blindness. Animal infections can also cause fetal diseases such as late abortions and neonatal death (Montoya and Liesenfeld 2004, Zhou et al. 2011, Stelzer et al. 2019).

Transmission of infection in animals and humans can be horizontal and vertical. Horizontal transmission includes ingestion of food (vegetables and fruits) and water contaminated by mature sporulated oocysts or consuming undercooked meat with tissue cysts (Jackson and Hutchison 1989). Vertical transmission occurs during late pregnancy, from an infected mother to the fetus through the placenta (Bigna et al. 2020).

Pigs are important food animals in China, where the amount of pork consumed exceeds that of beef, mutton, and other livestock meats. Pork is considered an important source of human infection for *T. gondii* because pigs are an important reservoir of the protozoa (Djokic et al. 2016, Pipia et al. 2018). Among animals naturally infected with *T. gondii*, pigs have the highest infection rates.

T. gondii infection rates encountered in some geographic locations of the country in the last few years show an epidemic trend: 10.1–53.4% in east China, 29.2–50.9% in north

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China, 8.0–35.4% in central China, 27.0% in south China, 22.7–71.9% in southwest China, 1.0–82.7% in northwest China, and 4.6–20.2% in northeast China (Yu et al. 2011, Zhou et al. 2010, 2011, Wang et al. 2012, 2019, Wu et al. 2012b, Chang et al. 2013, Xu et al. 2013a, Gu and Li 2014, Shao et al. 2014, Hua et al. 2016, Liu et al. 2016, Cai et al. 2017, Li et al. 2019).

Chongqing province is officially a municipality in Central China with five districts. It comprises the large city of Chongqing on the upper reaches of the Yangtze River and the large densely populated and rich agricultural land to the east of the city. It has a large pig industry, with pork production of 1,121,000 tons in 2019. In 2019, the per capita pork consumption for the province was 33.7 kg, ranking it among the highest in China (average 20.3 kg) (2020 China Statistical Yearbook). Although seroprevalence of *T. gondii* in slaughtered pigs in Chongqing has previously been reported (Tan et al. 2008, Wu et al. 2012a, Xu et al. 2013a,b), the current date is needed.

To understand the prevalence and risk factors for *T. gondii* in Chongqing, *Toxoplasma* antibodies were monitored in pig sera collected at different slaughterhouses comprising the five districts and distributed over the various seasons during the period 2015–2019.

Materials and Methods

Sample collection

Blood samples were randomly collected from seven swine slaughterhouses across five districts of Chongqing, namely Yubei District (YB-1 and YB-2), Jiangjin District (JJ-1 and JJ-2), Jiulongpo District (JLP), Nan'an District (NA), and Beibei District (BB) (Fig. 1). The slaughterhouses in Yubei District and Jiulongpo District are relatively larger than the others. A total of 1221 pig blood samples were collected from December 2015 to January 2019 and about 50 blood samples were collected from each slaughterhouse. These samples were also randomly spread across the spring, summer, autumn, and winter seasons.

Detection of T. gondii antibodies

Toxoplasma antibodies in pig sera were tested by using an enzyme-linked immunosorbent assay (ELISA) kit (QIAGEN, Leipzig, Germany), according to the manufacturer's instructions. Negative and positive control sera were included in the kit. The positive and negative samples were judged according to the following formula: $S/P = (MV\ OD_{sample} - MV\ OD_{NC})/(MV\ OD_{PC} - MV\ OD_{NC})$ where S/P = sample OD/mean OD of the Positive Control; MV = mean value; OD = optical density; PC = Positive Control; NC = Negative Control. The test was considered positive when $S/P \geq 0.3$, whereas $S/P < 0.3$ was considered to be negative.

Statistical analysis

The prevalence rates of *T. gondii* antibodies in different slaughterhouses, districts, seasons, and years were determined using Microsoft Excel 2017. Statistical analysis was done by IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY), Pearson's chi-squared (χ^2) test was used to analyze the differences, and logistic analysis was used to evaluate all of the potential risk factors. *p* Values of <0.05 were considered to be significant.

Ethics statement

The protocol of this work was approved by the Animal Care and Use of Chinese Academy of Agricultural Sciences, and authorized by the Animal Ethical Committee of Shanghai Veterinary Research Institute. All the blood samples obtained from slaughterhouses were permitted by the owners of

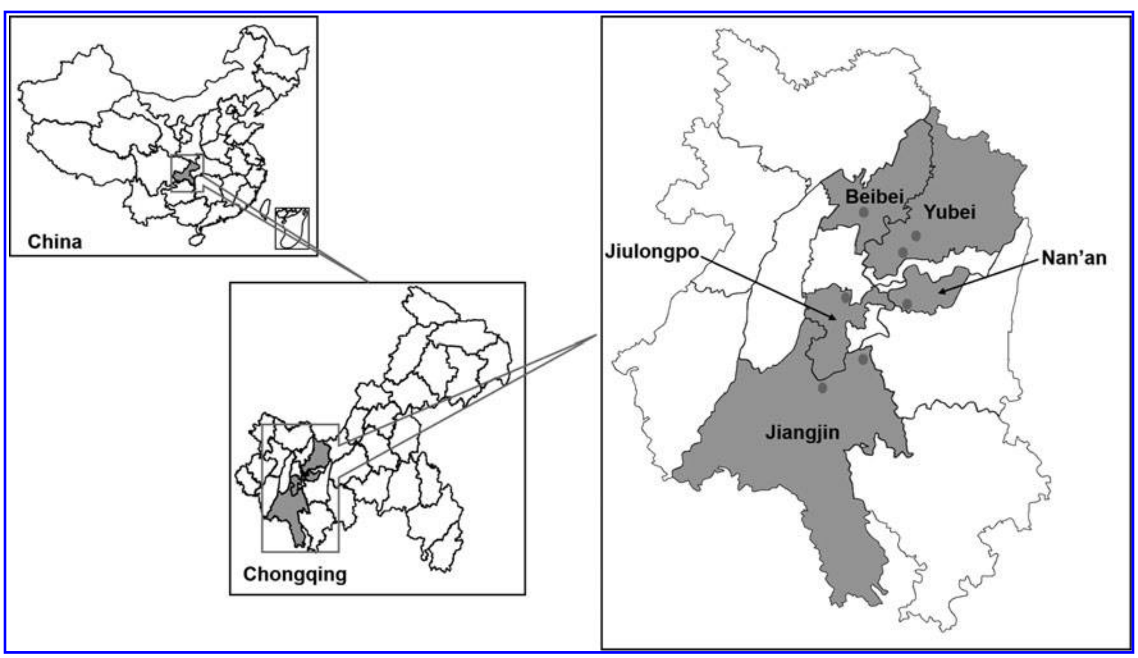

FIG. 1. Map of sampling districts of Chongqing in this study.

Table 1. Prevalence of *Toxoplasma gondii* from Different Slaughterhouses and Districts

Districts	*Slaughterhouses*	*No. of samples*	*No. of positives*	*Positive rates in different slaughterhouses (%)*	*95% CI*	*Positive rates in different districts (%)*	*95% CI*
Yubei	YB-1	384	28	8.0^{b}	5.4–11.4	9.8^{a}	7.8–12.2
	YB-2	415	47	11.3^{ab}	8.4–14.8		
Jiangjin	JJ-1	49	9	18.4^{a}	8.8–32.0	15.2^{a}	8.7–23.8
	JJ-2	50	6	12.0^{ab}	4.5–24.3		
Nan'an	NA	56	8	14.3^{ab}	6.4–26.2	14.3^{a}	6.4–26.2
Beibei	BB	53	7	13.2^{ab}	5.5–25.3	13.2^{a}	5.5–25.3
Jiulongpo	JLP	250	31	12.4^{ab}	8.6–17.1	12.4^{a}	8.6–17.1
Total		1221	136	11.1	9.4–13.0	11.1	9.4–13.0

Different lowercase letters within columns represent significant differences between slaughterhouses ($p < 0.05$).
CI, confidence interval.

slaughterhouses. During the whole experimental process, all the laboratory work on the study specimens was covered under the Animal Experimental Protocol of Shanghai Veterinary Research Institute (201008): ''Use of animal samples for the determination of zoonotic pathogen.''

Results

Prevalence of T. gondii *in pigs from different districts and slaughterhouses*

A total of 1221 serum samples were tested by ELISA. Of these, 136 were positive for *Toxoplasma* antibodies, and the total infection rate was 11.1%. Positive samples were found across the five districts, and the positive rate ranged from 9.8% (Yubei district) to 15.2% (Jiangjin district) across five districts, but there was no significant difference on positive rates among the different districts ($p > 0.05$) (Tables 1 and 4). For slaughterhouses, the highest infection rate was 18.4% (9/49) in JJ-1, and the lowest was 8.0% (28/348) in YB-1, with a significant difference ($p < 0.05$) (Tables 1 and 4).

Prevalence of T. gondii *infection in different seasons*

The seasonal seropositive rates of *T. gondii* infection from high to low were 14.6% (44/301) in autumn, 11.4% (35/306) in summer, 10.5% (32/305) in spring, and 8.1% (25/309) in winter. There was a significant difference in positive rates between autumn and winter ($p < 0.05$) (Tables 2 and 4).

Prevalence of T. gondii *infection in slaughter pigs in different years*

The positive rates of *T. gondii* in pig blood sera between different years ranged from 6.3% to 14.7%. The positive rates of *T. gondii* increased from 6.3% in 2015 to 14.7% in 2018, then declined to 10.1% in 2019, and 2018 and 2015 were significantly different ($p < 0.05$) (Tables 3 and 4).

Discussion

Although epidemiological investigations on the *T. gondii* infection in slaughterhouse pigs are common in different countries (Dubey et al. 2020), there are few reports in Chongqing. Pork is a popular meat in Chongqing, and residents there have the largest per capita pork consumption in China.

Although previous studies have reported *T. gondii* infection in pigs in different districts of Chongqing, the positive rates are very high, with 60.4% (Tan et al. 2008), 71.9% (Xu et al. 2013b), and 76.0% (Xu et al. 2013a), respectively. Therefore, the prevalence of *T. gondii* in pigs in Chongqing is not clear. In this study, the seroprevalence and potential risk factors for *T. gondii* in slaughterhouse pigs in Chongqing during a recent 5-year period from 2015 to 2019 were determined.

A total positive rate of 11.1% was detected for the five districts of Chongqing, which was lower than the other seroprevalence rates of *T. gondii* in the world except for Europe (6.3%), such as 12.9% in North America, 32.6% in Africa, 29.1% in South America, 21.4% in the rest of Asia, and 32.9% in China (Dubey 2010, Dong et al. 2018). These differences may be due to the location and the environment, temperature, climate, landscape, and other geographical factors.

Compared with previous reports on Chongqing, the prevalence rates of the present study were much lower than those of Tan et al. (60.4%), Xu et al. (71.9%), and Wu et al. (30.6%) (Tan et al. 2008, Wu et al. 2012a, Xu et al. 2013b). It could be because that control of *T. gondii* infection in pig farms has improved in recent years. In addition, compared with the surrounding cities, the results of this study are higher than those of Henan (9.9% tested by modified agglutination test) and Hunan (8.0% tested by indirect hemagglutination [IHA]), and lower than those of Shaanxi (19.9% tested by IHA), Sichuan (29.5% tested by IHA), Guizhou (70.0% tested by ELISA), and Hubei (24.5% tested by ELISA) (Shu et al. 2011, Tao et al. 2011, Li et al. 2015, Wu et al. 2017, Wang et al. 2019, Su et al. 2020).

These differences may be explained by differences in the detection methods or the age of animals, because different ELISA kits were used in these studies, and some samples were collected from different age groups in pig farms, while all the samples were collected from slaughterhouses (nearly 200 days old per pig) in this study.

Table 2. Prevalence of *Toxoplasma Gondii* in Different Seasons

Season	*No. of samples*	*No. of positives*	*Positive rates (%)*	*95% CI*
Spring	305	32	10.5^{ab}	7.3–14.5
Summer	306	35	11.4^{ab}	8.1–15.6
Autumn	301	44	14.6^{a}	10.8–19.1
Winter	309	25	8.1^{b}	5.3–11.7
Total	1221	136	11.1	9.4–13.0

Different lowercase letters within columns represent significant differences between groups ($p < 0.05$).

Table 3. Prevalence of *Toxoplasma gondii* Infection in Slaughter Pigs in Different Years

Sampling years	*No. of samples*	*No. of positives*	*Positive rates (%)*	*95% CI*
2015	160	10	6.3^{b}	3.0–11.2
2016	305	31	10.2^{ab}	7.0–14.1
2017	307	36	11.7^{ab}	8.4–15.9
2018	300	44	14.7^{a}	10.9–19.2
2019	149	15	10.1^{ab}	5.7–16.1
Total	1221	136	11.1	9.4–13.0

Different lowercase letters within columns represent significant differences between groups ($p < 0.05$).

Among the different districts studied here, the Jiangjin District had the highest seroprevalence rate of 15.2%, but it was not significantly different from the others ($p > 0.05$). This result did not find region to be a risk factor of *T. gondii* infection, and was similar to the findings of Li et al. (2019), who did not find region as a key factor for *T. gondii* infection in free-ranging pigs in six regions of northeast China.

Zhang et al. (2020), however, reported that different regions were the main factors affecting *T. gondii* infection in Shanghai. Logistic regression analysis showed that slaughterhouse was not a potential risk factor for *T. gondii* ($p < 0.05$) infection in pigs in the present study, but there was a significant difference between JJ-1 and YB-1 with Pearson's chi-square tests. This was possibly due to the small sample sizes from JJ-1 slaughterhouses. In this study, samples from JJ-1 were collected only one time, while samples from YB-1 were collected at least two times every year. Single sampling will reduce the accuracy of the results, and so, the results need to be confirmed by increasing the number of samples collected in JJ-1 in the future.

Table 4. The Potential Risk Factor of *Toxoplasma gondii* in Pigs in Chongqing

Characteristics		*OR (95% CI)*	p
Districts	Yubei (YB)	0.8 (0.5–1.1)	0.250
	Jiangjin (JJ)	1.3 (0.6–2.5)	0.494
	Nan'an (NA)	1.2 (0.5–2.7)	0.702
	Beibei (BB)	1.1 (0.4–2.6)	0.872
	Jiulongpo (JLP)	*Reference*	
Slaughterhouses	YB-1	*Reference*	
	YB-2	0.6 (0.4–1.1)	0.80
	JJ-1	0.9 (0.6–1.5)	0.677
	JJ-2	1.6 (0.7–3.6)	0.265
	NA	1.0 (0.4–2.3)	0.937
	BB	1.2 (0.5–2.7)	0.702
	JLP	1.1 (0.4–2.6)	0.872
Season	Spring	1.3 (0.8–2.3)	0.307
	Summer	1.5 (0.9–2.5)	0.164
	Autumn	1.8 (1.2–3.3)	0.012*
	Winter	*Reference*	
Years	2015	*Reference*	
	2016	1.7 (0.8–3.6)	0.161
	2017	2.0 (1.0–4.1)	0.064
	2018	2.6 (1.3–5.3)	0.009**
	2019	1.7 (0.7–3.9)	0.223

*Significant difference ($p < 0.05$); **significant difference ($p < 0.01$).

OR, odds ratio.

In this study, the highest positive rate was found in autumn (14.6%) and the lowest was in winter (8.1%), and the difference was statistically significant ($p < 0.05$). This result was similar to Zhang et al. (2020) who reported that the highest seropositive rate of *T. gondii* in slaughterhouse pigs in Shanghai was in autumn (17.8%) and the lowest in winter (6.7%). Li et al. (2019) also found that the serum-positive rates of *T. gondii* in autumn (28.2%) and summer (21.9%) were higher than that in winter (15.9%) in free-range pigs in northeast China, whereas the opposite results occurred in pigs at the growth stage in Hunan and in breeding sows in Fujian, where the highest infection rates were found in winter and spring, respectively (Huang et al. 2010, Wang et al. 2019).

All these results suggest that *T. gondii* infection in different seasons may be influenced by the climatic conditions of different cities. Logistic regression analysis showed that season was a potential risk factor, and the risk of serum being positive for *T. gondii* in pigs in autumn is 1.8 times (odds ratio [OR] = 1.8, confidence interval [95% CI] = 1.2–3.3, $p < 0.05$) higher than that in winter. Some studies showed that the infection rate of *T. gondii* in pigs was high in tropical climate areas (Alvarado-Esquivel et al. 2014, Hou et al. 2018, Zhang et al. 2019, 2020).

Moreover, we found that the infection rate ranged from 6.3% to 14.7% from 2015 to 2018, and then decreased in 2019. The differences in *T. gondii*-positive rates were not obvious except between the years 2018 and 2015 ($p < 0.01$). These positive rates nevertheless are lower than those reported previously by Xu et al. (2013a), who detected positive rates ranging from 51.8% to 87.6% between 2004 and 2011 in 11,700 serum samples from 39 counties and districts in Chongqing.

This difference may be due to the emergence of the porcine reproductive and respiratory syndrome, swine fever, and other diseases in this district in recent years, which made most pig farms strengthen the management, prevention, and control of the diseases, and increase public health awareness in pig production. Logistic regression analysis showed that year was the other potential risk factor and the risk of serum being positive for *T. gondii* in pigs in 2018 was 2.6 times (OR = 2.6, 95% CI = 1.3–5.3, $p < 0.01$) higher than that in 2015, and this result is similar to the results of Li et al. (2019).

Conclusions

In this study, 1221 pig serum samples were collected continuously from seven slaughterhouses in five districts of Chongqing from 2015 to 2019 and analyzed for *T. gondii* antibodies by ELISA. The results showed a seropositive rate for *T. gondii* of 11.1%. Season and year were identified as potential risk factors for the infection. To the best of our knowledge, there are no data on the detection of *T. gondii* antibody in pig sera in Chongqing in recent years. This study can enrich the serological data of *T. gondii* infection in pigs in Chongqing, invaluable for pork safety and toxoplasmosis prevention and control in China.

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Author Disclosure Statement

No conflicting financial interests exist.

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