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Seroprevalence of *Toxoplasma gondii* infection in people in southeast Hubei province, China

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Abstract. *Toxoplasma gondii* is a world-widely spread zoonotic parasite. However, scarce knowledge is known about the prevalence of *T. gondii* infection in people in Hubei province, China. This study herein was to perform epidemiological investigation of *T. gondii* infection in people in this region. A total 12527 blood samples were obtained during 2015-2018, and were assayed for *T. gondii* antibodies of IgG and IgM, respectively by employing an indirect hemagglutination test (IHA). The results discovered that the prevalence of *T. gondii* in people was 2.44% and 6.1%, respectively based on antibodies of IgG and IgM, respectively. The prevalence was ranged from 0.3% to 5.4% during 2015-2018 based on IgM antibodies. For genders, the prevalence was 0.7% and 2.6% in males and females, respectively based on IgM antibodies. In different years, the prevalence was ranged from 4.9% to 14.0% based on IgG antibodies. The prevalence of *T. gondii* was 4.9% and 6.6% in males and femalesy based on IgG antibodies. The current results may be helpful for the implementation of preventive measures against *Toxoplasma* infection among people living in this region.

INTRODUCTION

Toxoplasmosis is a zoonotic parasitic disease in mammals and animals caused by *Toxoplasma gondii* (Li *et al*., 2014). This protozoan parasite can develop cysts in the host bodies, especially in muscles and brains, which can live for years or even lifelong (Pappas *et al*., 2009). *T. gondii* can infect a wide range of cold-blooded and warm-blooded animals, including 45 species of mammals (Sukthana, 2006), 70 species of birds (Weiss and Dubey, 2009), and five species of reptiles (Sukthana, 2006; Weiss and Dubey, 2009). For human beings, *T. gondii* infection occurs mainly through the ingestion of food and related products (fish, sausage, canned meat, dairy, cream, eggs, etc.) from infected animals or polluted by this protozoan (Wang *et al*., 2013). The majority of adults infected with *T. gondii* are recessive infections, while infants (el-Sagaff *et al*., 2005), pregnant women (Pappas *et al*., 2009) and immunosuppressive persons (Pappas *et al*., 2009) infected with this protozoa can cause abortion, stillbirth, encephalitis, etc., with high mortality and serious harm (Zhang *et al*., 2012; Li *et al*., 2017).

At present, the detection of *T. gondii* infection is mainly done through serological methods (Meroni and Bollani, 2003; Filisetti *et al*., 2015; Pomares and Montoya, 2016), including antigens or antibodies detection.

The main antigens used are soluble antigens (cytoplasmic antigens) and membrane antigens. The antibody induced by soluble antigens (cytoplasmic antigens) appeared earlier (detected by staining test and indirect immunofluorescence test), while the antibody induced by membrane antigens appeared later (detected by indirect hemagglutination test, etc.). Multiple methods can be used to improve the detection rate.

T. gondii infection is distributed globally and the population is generally susceptible to infection (Yarovinsky, 2014). However, the infection rate varies from place to place, with the general infection rate ranging from 25% to 50% and the highest reaching over 80% (Lehmann *et al*., 2006). Serum antibody positive rate in the UK was 20% – 40% (Villena *et al*., 2010), the United States was 50% to 60% (Villena *et al*., 2010), French was 80% – 90% (Zhou *et al*., 2011), 20% to 30% in Japan (Zhou *et al*., 2011), China was 5% - 20% (Liandi and Qixu, 1996). Previous survey preformed by Chinese medical workers showed that the average seroprevalence of *T. gondii* was 5.84% in China (Enshu, 1992). The prevalence of *T. gondii* varied from regions to regions in China (Yuancong and Junzhao, 1994).

Although there have been many scientific researchers and medical workers investigated the prevalence of *T. gondii* in many regions of China, but those investigation data was limited in short time and number of samples. Here, we detected the serum specific IgM and IgG antibodies of *Toxoplasma* infection to investigate the prevalence of this protozoan in population in Hubei province, China in the past four years (2015-2018).

MATERIALS AND METHODS

Ethical approval and consent to participate

This study was performed under the instructions and approval of the ethics committee of Huangshi Central Hospital, Affiliated Hospital of Hubei Polytechnic University (Permit no. 2019-YXY-016).

Sample Collection

All the venous blood samples were collected from normal people in the area of southeast Hubei province, China (Fig. 1). There were 2115 blood samples (1901 samples for IgM detecting and 214 samples for IgG detecting) in 2015, 3693 blood samples (2906 samples for IgM detecting and 787 samples for IgG detecting) in 2016, 3891 blood samples (2925 samples for IgM detecting and 966 samples for IgG detecting) in 2017, 2828 blood samples (2135 samples for IgM detecting and 693 samples for IgG detecting) in 2018. After collection, all the blood samples were centrifuged at 3,000 g for 5 minute, and serums were separated and stored at -4°C for further analysis.

Determination of IgM and IgG antibodies Against *T. gondii*

Serum samples were tested for IgM and IgG antibodies against *Toxoplasma* infection by using a commercial antibody detection kit for anti-*Toxoplasma* IgM (TOXO IgM) and IgG (TOXO IgG) (Purchased from Shenzhen new Industry Biomedical Engineering Co. LTD, Shenzheng, China) according to the manufacturer's instructions. The detail steps of commercial antibody detection kit (TOXO IgG and IgM) are as follows.

After collecting the blood, 1000×g centrifugation was performed for 10 minutes to rapidly and carefully separate the serum from the red blood cells. The sample was diluted by 1:1 and then was added into the reaction well, the standard sample and blank control were set at the same time. Add the standard and the target samples into the reaction well to be tested. Immediately add enzyme conjugate, cover the membrane plate, gently shake the mixture, and incubate at 37°C for 1 hour. Shake off the liquid inside the well and rinse with washing liquid for 3 times. Add affinity enzyme-HRP into each well, vibrate gently and mix well, incubate at 37°C for 30 minutes. Shake off the liquid inside the well and rinse with washing liquid for 3 times. Substrates A and B were added to each well and the mixture was shaken gently, and incubated at 37°C for 10 minutes. Avoid light. Take out the enzyme label plate,

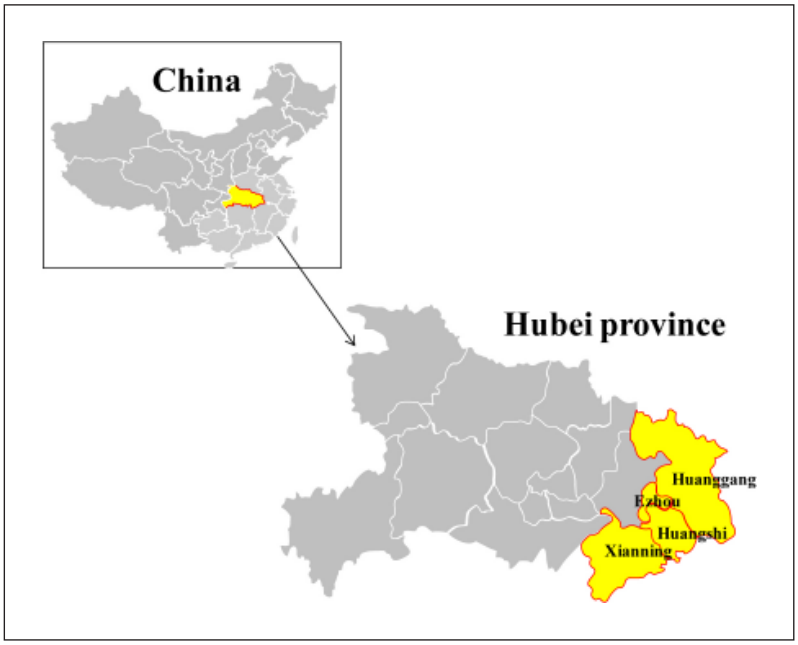

Figure 1. Geographical distribution of sample collection.

add termination solution immediately. The OD results determined immediately in the 450 nm wavelength after the addition of the termination solution.

Statistical Analysis

Statistical analysis was performed by chi-square test with SPSS (Statistical Analysis System, Version 23.0) software. The differences were considered statistically significant when $P<0.05$.

RESULTS

The prevalence of *T. gondii* infection was 2.44% based on IgM antibodies. The prevalence was ranged from 0.3% to 5.4% during 2015-2018. The prevalence in 2015 ($P<0.001$), 2016 ($P<0.001$), 2017 ($P<0.001$) was uncovered to be obviously higher than that in 2018, respectively. In genders, the prevalence was 0.7% and 2.6% in males and females, respectively, with significant difference between genders during 2015-2018. Significant difference was also found in the prevalence of *T. gondii* in males and females in year of 2015 ($P<0.05$) and 2016 ($P<0.05$), respectively (Table 1).

A total 164 out of 2687 (6.1%) samples were tested to be positive against IgG antibodies of *T. gondii* during 2015-2018. In different years, the prevalence was ranged from 4.9% to 14.0%. The prevalence of *T. gondii* in 2015 was found to be obviously higher than that in 2017 ($P<0.001$). The prevalence of *T. gondii* was 4.9% and 6.6% in males and females, respectively. Significant difference was found in the prevalence of *T. gondii* in males and females in the year of 2015 ($P<0.05$), respectively (Table 2).

DISCUSSION

T. gondii is an important zoonotic parasitic disease and enough attention should be paid word-widely (Li *et al.*, 2017). So, it is of great importance and very meaningful to monitor the prevalence of *T. gondii* in people periodically. According to the current results, the total prevalence in 4 years (from 2015 to 2018) of *T. gondii* infection in people was 6.1% and 2.44%, respectively based on

Table 1. The seroprevalence of *T. gondii* in population by Chemiluminescence test in the area of southeast Hubei province, China based on IgM antibodies

Year	Male		Female		Total	
	No. positive/ Samples	Sero-prevalence (%)	No. positive/ Samples	Sero-prevalence (%)	No. positive/ Samples	Sero-prevalence (%)
2015[d]	2/172	1.2	100/1729	5.8[a]	102/1901	5.4
2016[e]	2/298	0.7	84/2608	3.2[b]	86/2906	3
2017[f]	1/309	0.3	40/2616	1.5	41/2925	1.4
2018	3/359	0.8	4/1776	0.2	7/2135	0.3
Total	8/1138	0.7	228/8729	2.6[c]	236/9687	2.44

[a]Differences between the genders were found statistically significant in 2015 ($P<0.05$, $\chi^2 = 6.579$).
[b]Obvious differences between the genders were found in 2016 ($P<0.05$, $\chi^2 = 6.054$).
[c]Differences between the genders were found statistically significant during 2015-2018 ($P<0.001$, $\chi^2 = 15.715$).
[d]The prevalence of *T. gondii* in populationin 2015 was found to be significantly higher than the prevalence in 2018 ($P<0.001$, $\chi^2 = 97.121$).
[e]The prevalence of *T. gondii* in populationin 2016 was found to be significantly higher than the prevalence in 2018 ($P<0.001$, $\chi^2 = 47.067$).
[f]The prevalence of *T. gondii* in populationin 2017 was found to be significantly higher than the prevalence in 2018 ($P<0.001$, $\chi^2 = 15.146$).

Table 2. Seroprevalence of *T. gondii* in population by chemiluminescence test in the area of southeast Hubei province, China based on IgG antibodies

Year	Male		Female		Total	
	No. positive/ Samples	Sero-prevalence (%)	No. positive/ Samples	Sero-prevalence (%)	No. positive/ Samples	Sero-prevalence (%)
2015[h]	3/53	5.6	27/161	16.8[g]	30/214	14
2016	14/250	5.6	37/537	6.9	51/787	6.5
2017	11/252	4.4	36/741	4.9	47/966	4.9
2018	13/279	4.7	23/414	5.6	36/693	5.2
Total	41/834	4.9	123/1853	6.6	164/2687	6.1

[g]Differences between the genders were found statistically significant in 2015 ($P<0.05$, $\chi^2 = 4.083$).
[f]The prevalence of *T. gondii* in population in 2015 was found to be remarkably higher than the prevalence in 2017 ($P<0.001$, $\chi^2 = 24.064$).

antibodies of IgG and IgM. The prevalence in Hubei was lower than the prevalence in Tibetans in Tibet (10.7%) (Xu *et al.*, 2015), people in Guizhou (15.1%) (Li *et al.*, 2017), and psychiatric patients in Zhejiang (13.3%) (Chen *et al.*, 2019). The difference might be due to the eating culture, hygiene condition, socioeconomic status (Li *et al.*, 2017).

The infected people may transmit this important and serious parasite to animals and other people, as nowadays there is a growing trend of pets, especially cats keeping in China. For most people, the infection of *T. gondii* maybe asymptomatic, but for people such as pregnant women and transplant people (el-Sagaff *et al.*, 2005; Pappas *et al.*, 2009; Feitosa *et al.*, 2014), *T. gondii* may cause serious harm to those people. Human beings infect *T. gondii* mainly through the ingestion of raw meat and polluted water contaminated with this parasite (Li *et al.*, 2014). The present study depicted that the prevalence of *T. gondii* infection in Hubei showed a drop trend since 2015 based on the data of antibodies of IgM and IgG (Table 1 & 2), which may refer to the effect of the control and management of pets in the past few years, as cat and felid are the

definitive host of *T. gondii* (Li *et al*., 2014; Li *et al*., 2017). However, more attention should be paid to this harmful parasite, as *T. gondii* not only causes serious economic loss and public problems, but also the ability of develop high resistance anti-parasitic drugs (Wedrychowicz *et al*., 2015). In the current study, a higher prevalence of *Toxoplasma* infection was found in females (IgG antibodies) compared to males counterpart which may lead to serious harm to fetus (el-Sagaff *et al*., 2005; Pappas *et al*., 2009).

In conclusion, the current study reported for the first time about the prevalence of *T. gondii* infection in people in the southeast Hubei province, China. The current results may be helpful for the implementation of preventive measures against *Toxoplasma* infection in the population in this region.

Authors' Contributions

Experiments design: Shen, Zh. Zh., Wang, H.L. and Luo, H.Q. Experiments performance: Shang, X.L. and Hu, F. Data analysis: Li, K. and Li, Z.J. Manuscript preparation: Shen, Zh. Zh. and Zhou, W.J. All authors read and approved the final manuscript.

Consent for Publication

Not applicable.

Conflict of Interest Statement

The authors state that there are no conflicts of interest.

Disclosure

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