Seroprevalence of Toxoplasma gondii Among Primary School Children in Shandong Province, China

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Abstract: Although Toxoplasma gondii infection in primary school children has been investigated in many countries, limited surveys have been available in primary school children in China. In the present study, we report the seroprevalence of T. gondii infection in primary school children in Shandong province, China. Sera from 6,000 primary school children were evaluated for T. gondii antibodies with ELISA. The overall seroprevalence of T. gondii infection was 16.0% (961/6,000), of which 14.5% (870/6,000) were positive for anti-T. gondii IgG antibodies, 3.4% (206/6,000) positive for IgM, and 1.9% (115/6,000) were positive for both IgG and IgM. The results of the present investigation indicated a high seroprevalence of T. gondii infection in primary school children in Shandong province, China. Therefore, effective measures should be taken to prevent and control T. gondii infection in primary school children in this province. To the best of our knowledge, this is the first report of T. gondii seroprevalence in primary school children in Shandong province, China.

Key words: Toxoplasma gondii, seroprevalence, primary school children, ELISA, China

Toxoplasmosis is an important parasitic zoonosis caused by the protozoan Toxoplasma gondii, which is widespread in animals and humans worldwide [1]. Nearly one-third of the world population has been infected with this parasite, and in China, about 7.9% of the population were reported to be exposed to T. gondii with a sustained growing number in recent years [2]. Humans are infected by ingesting oocysts shed by cats or consuming undercooked meat with parasite tissue cysts. Almost infected adults are usually asymptomatic, but blindness and mental retardation can be caused in congenitally infected children and severe diseases in those with compromised immunity [3]. To date, toxoplasmosis continues to be a significant public health problem around the world, and no commercial vaccines are available, and treatment relies on chemical drugs.

T. gondii antibodies are indicative of infection, and that infection is long-lasting (generally thought to last throughout life). IgM and IgG are the 2 specific antibodies that have their presence in the sera, and reveal the stage and kind of infection to T. gondii. It has been shown that IgM antibodies are usually known as a marker of acute infection that appear earlier and decrease more quickly than IgG antibodies and are frequently the first class of antibodies detected after primary infection [4]. However, in acute infection stage, IgG may not be produced. If detection of T. gondii infection relies only on IgG, some positive samples may be neglected. So, the diagnosis of recently acquired toxoplasmosis is generally based on the detection of specific IgM antibodies, followed by detecting the specific IgG antibodies.

Although the seroprevalence of T. gondii infection in primary school children (6-11 years old) has been reported in many countries around the world [5-8], little is known about the seroprevalence of T. gondii infection in primary school children in China [9,10]. However, these literatures were published in Chinese language in local journals and are not readily accessible to international readers. More importantly, there has been no report of T. gondii infection in primary school children in Shandong province, China. Therefore, the objective of the present investigation was to examine the T. gondii seroprevalence in primary school children in Shandong province, China. The results should provide base-line data for recommendations with regards to prevention and control of toxoplasmosis in this region and elsewhere.

A total of 6,000 blood samples were collected from primary
school children in hospitals which are distributed in 15 representative administrative regions (Fig. 1) in Shandong province between September 2012 and October 2014. The primary school children were 6-11 years old. Children who participated in health screenings in the hospitals were recruited. The purpose and procedures of the study were explained to all participants, and written informed consent was obtained from all of them. This study was approved by the Institutional Review Board (IRB) of the Institute of Biomedicine at Qingdao University (approval no: 2011013). Parents/guardians provided informed consent on behalf of all child participants. The sera were collected with agreement from the volunteers. Blood samples were then centrifuged at 1,000 g for 10 min, and serum was obtained, frozen, and stored at -20°C until use.

The ELISA kits (Demeditec Diagnostics GmbH, Kiel-Wellsee, Germany) was performed according to the manufacturer’s instructions. This ELISA kit has been extensively used for detecting specific IgG and IgM antibodies to T. gondii in humans and other mammals for many years [11-13]. Serum samples were diluted to 1:500. The optical density (OD) values were read using an automated microplate reader (Bio-Tek, Winooaski, Vermont, USA). The threshold value was determined by the mean of 3 critical controls in each test. A result equal to or greater than threshold values was considered positive. The IgG test kit has reported sensitivity and specificity of 98% and 99%, respectively, and the IgM test kit has reported sensitivity and specificity of 100% and 99%, respectively [14].

The data were analyzed statistically using the PASW Statistics 18 (IBM Corporation, Somers, New York, USA); 95% confidence intervals (CI) are given. The value of $P<0.05$ differences between levels within factors and interactions were considered to be statistically significant.

Primary school children are particularly vulnerable to toxoplasmosis due to their habits of playing in water, soil, eating various raw foods, or contact with pets, including dogs, cats, and birds and hence they are an ideal target group to investigate T. gondii prevalence. Data collected from this age group can thus be used to assess whether T. gondii threatens the health of school-aged children, and also as a reference for evaluating the need for community interventions. Shandong province is a subtropical region; climatic and living conditions favor the surveillance of many parasites, including T. gondii. However, systemic studies about seroprevalence of T. gondii infection in primary school children in this province remain largely unclear to date.

The overall seroprevalence of T. gondii infection was 16.0% (961/6,000), of which 14.5% (870/6,000) were positive for anti-T. gondii IgG antibodies, 3.4% (206/6,000) positive for IgM, and 1.9% (115/6,000) were positive for both IgG and IgM. Antibodies against T. gondii were detected in 16.0% primary school children, which is slightly higher than those in Iran [5] and other provinces of China [9,10], but was significantly lower than those in West Africa and Marshall Islands [6,7]. This is most likely due to difference in geographical conditions, dietary habit and lifestyle. Another reason for the different seroprevalence may be due to using different investiga-
Table 1. Seroprevalence of *Toxoplasma gondii* infection in primary school children in Shandong province, China

| City       | No. tested | T. gondii IgG seropositive | T. gondii IgM seropositive | Both seropositive | Total seropositive |
|------------|------------|----------------------------|----------------------------|-------------------|--------------------|
|            |            | No. posit. | Prevalence (%) | No. posit. | Prevalence (%) | No. posit. | Prevalence (%) | No. posit. | Prevalence (%) |
| Zibo       | 378        | 73         | 19.3           | 16         | 4.2           | 6          | 1.6           | 83          | 2.2           |
| Zaozhuang  | 199        | 39         | 19.6           | 11         | 5.5           | 5          | 2.5           | 45          | 2.2           |
| Dongying   | 220        | 32         | 14.5           | 11         | 5             | 3          | 1.4           | 40          | 2.1           |
| Jinan      | 330        | 54         | 16.4           | 9          | 2.7           | 4          | 1.2           | 59          | 2.1           |
| Taian      | 315        | 33         | 10.5           | 12         | 3.8           | 6          | 1.9           | 39          | 1.4           |
| Weihai     | 307        | 41         | 13.4           | 7          | 2.3           | 7          | 2.3           | 41          | 1.4           |
| Rizhao     | 218        | 32         | 14.7           | 12         | 5.5           | 7          | 2.7           | 37          | 1.4           |
| Binzhou    | 269        | 33         | 12.3           | 14         | 5.2           | 9          | 3.3           | 38          | 1.4           |
| Dezhou     | 378        | 44         | 11.6           | 16         | 4.2           | 8          | 2.1           | 52          | 1.7           |
| LiaoCheng  | 625        | 53         | 8.5            | 19         | 3             | 9          | 1.4           | 63          | 1.3           |
| Heze       | 490        | 48         | 9.8            | 14         | 2.9           | 14         | 2.9           | 48          | 9.8           |
| Laizhou    | 568        | 59         | 10.4           | 14         | 2.5           | 8          | 1.8           | 65          | 11.4          |
| Linyi      | 435        | 64         | 14.7           | 17         | 3.9           | 9          | 2.1           | 72          | 16.6          |
| Jinan      | 468        | 112        | 23.9           | 16         | 3.4           | 8          | 1.7           | 120         | 25.6          |
| Qingdao    | 800        | 153        | 19.1           | 18         | 2.3           | 12         | 1.5           | 159         | 19.9          |
| Total      | 6,000      | 870        | 14.5           | 206        | 3.4           | 115        | 1.9           | 961         | 16            |

Table 2. Prevalence of *Toxoplasma gondii* infection in primary school children in different sex and age groups in Shandong province, China

| Factor | Category | No. tested | T. gondii IgG seropositive | T. gondii IgM seropositive | Both seropositive | Total seropositive |
|--------|----------|------------|----------------------------|----------------------------|-------------------|--------------------|
|        |          |            | No. posit. | Prevalence (%) | No. posit. | Prevalence (%) | No. posit. | Prevalence (%) | No. posit. | Prevalence (%) | P-value |
| Sex    | Male     | 3,498      | 608        | 17.4           | 125       | 3.6           | 68        | 1.9           | 665       | 19.0           | 0.629   |
|        | Female   | 2,502      | 262        | 10.5           | 81        | 3.2           | 47        | 1.9           | 296       | 11.8           |        |
| Age (yr) | 6-7   | 1,687      | 194        | 11.5           | 52        | 3.1           | 29        | 1.7           | 217       | 12.9           | 0.752   |
|        | 8-9     | 1,803      | 255        | 14.1           | 61        | 3.4           | 32        | 1.8           | 284       | 15.6           |        |
|        | 10-11   | 2,510      | 421        | 16.8           | 93        | 3.7           | 54        | 2.2           | 460       | 18.3           |        |

It is generally known that no gender difference is usually found in T. gondii prevalence [15]. The overall seroprevalence of T. gondii infection in male primary school children (19.0%; 665/3,498) was higher than that in female primary school children (11.8%; 296/2,502) (Table 2), but there was no significant difference in seroprevalence between male and female primary school children (P > 0.05). This result was consistent with that of a recent study from Shandong and Jilin provinces of China [4]. It might be due to boys or girls having similar routes for acquisition of T. gondii infection through frequent contact with risk factors, including dogs, cats, playing in the soil, eating raw/undercooked meats, and eating raw vegetables [16]. Importantly, young girls are infected in their childhood as they are not at risk to transmission of T. gondii at the present stage, but T. gondii infection can causes transplacental transmission from them to their offspring when they will be pregnant in adult ages. A previous study has indicated that T. gondii infection in 80 puerperas and their newborn babies showed that the seroprevalence were 8.8% and 6.3%, respectively. The vertical transmission was 70% by using ELISA, which was still serious in China [2]. So, the findings from the present study need to pay much attention to young girls, although most infected young girls have no apparent serious illness at this stage.

It is acknowledged that the seroprevalence increases with age as shown in data from various countries [6]. A hypothesis would be that the increase is a reflection of increasing exposure years as the children get older [17]. The overall seroprevalence of T. gondii in primary school children increased progressively with age (Table 2), and the prevalence (18.3%) in older primary school children (10-11 year-old) was higher than that in 8-9 year-old (15.6%) and 6-7 year-old (12.9%) primary school...
children (P > 0.05), indicating that older primary school children were more likely to be seropositive than young primary school children under 10-year-old, consistent with that of previous studies [9,10]. The results provided further evidence for the increased risk of Toxoplasma gondii infection with acquisition of age.

Felids play a vital role in the transmission of T. gondii as the only definitive hosts of the parasite. Infected cats are considered to be a potential threat to public health because they can shed and excrete environmentally resistant oocysts in their feces [18]. In addition, soil contact has been strongly associated with T. gondii infections, as the suspected source of outbreaks was the oocysts from soil [15]. Moreover, a recent study showed that raising cats was estimated to be a risk factor associated with T. gondii seroprevalence in children in China [4]. However, our study also has some limitations that we did not perform examinations of T. gondii oocysts from soil in public schools and parks in the local area. Therefore, further studies are necessary to estimate the prevalence of T. gondii oocysts in soil in public schools and parks in the local area.

In conclusion, the present results indicated a high seroprevalence of T. gondii infection in primary school children in Shandong province, China. Therefore, integrated and improved strategies should be implemented to prevent and control T. gondii infection in primary school children in this region, especially among young girls. To the best of our knowledge, this is the first report of T. gondii seroprevalence in primary school children in Shandong province, China.

CONFLICT OF INTEREST

We have no conflict of interest related with this report.

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