

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

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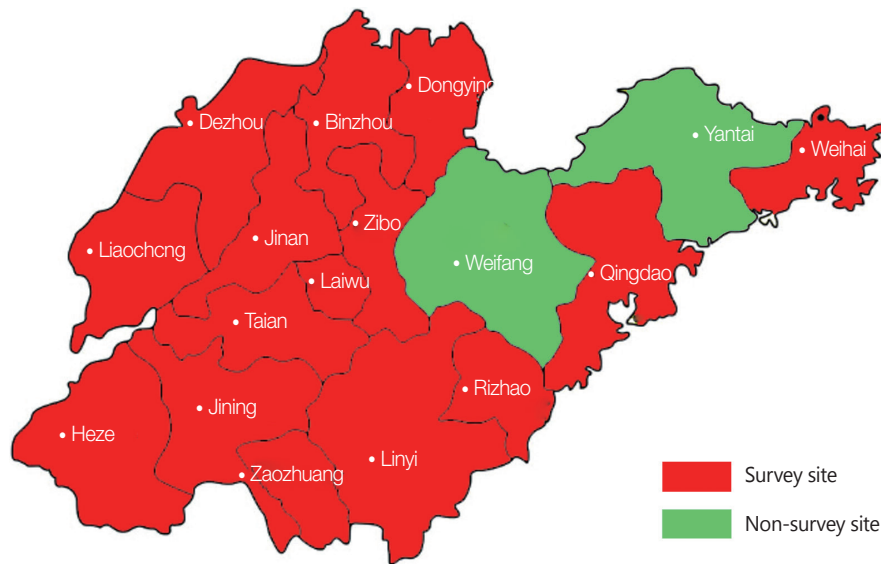


Fig. 1. The sampling locations for *Toxoplasma gondii* in different regions of Shandong province, China.

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, Winooski, 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

	No. tested	<i>T. gondii</i> IgG seropositive		<i>T. gondii</i> 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	22
Zaozhuang	199	39	19.6	11	5.5	5	2.5	45	22.6
Dongying	220	32	14.5	11	5	3	1.4	40	18.2
Jining	330	54	16.4	9	2.7	4	1.2	59	17.9
Taian	315	33	10.5	12	3.8	6	1.9	39	12.4
Weihai	307	41	13.4	7	2.3	7	2.3	41	13.4
Rizhao	218	32	14.7	12	5.5	7	3.2	37	17
Binzhou	269	33	12.3	14	5.2	9	3.3	38	14.1
Dezhou	378	44	11.6	16	4.2	8	2.1	52	13.8
Liaocheng	625	53	8.5	19	3	9	1.4	63	10.1
Heze	490	48	9.8	14	2.9	14	2.9	48	9.8
Laiwu	568	59	10.4	14	2.5	8	1.4	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	<i>T. gondii</i> IgG seropositive		<i>T. gondii</i> IgM seropositive		Both seropositive		Total seropositive		P-value
			No. posit.	Prevalence (%)	No. posit.	Prevalence (%)	No. posit.	Prevalence (%)	No. posit.	Prevalence (%)	
Sex	Male	3,498	608	17.4	125	3.6	68	1.9	665	19	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	

tional methods. *T. gondii* seroprevalence in primary school children from different regions ranged from 9.8 to 25.6% (Table 1), having no statistically significant differences ($P > 0.05$).

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 cause 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 *T. 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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