

Serologic Survey of Toxoplasmosis in Seoul and Jeju-do, and a Brief Review of Its Seroprevalence in Korea

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Abstract: Knowledge of the prevalence of human *Toxoplasma gondii* infection is required in the Republic of Korea. In this study, we surveyed the seroprevalence of *T. gondii* infection and analyzed the risk factors associated with seropositivity among residents in 2 administrative districts; Seoul and the island of Jeju-do, which have contrasting epidemiologic characteristics. Sera and blood collected from 2,150 residents (1,114 in Seoul and 1,036 in Jeju-do) were checked for IgG antibody titers using ELISA and for the *T. gondii* B1 gene using PCR. In addition, participants completed a questionnaire that solicited information on gender, age, occupation, eating habits, history of contact with animals, and travel abroad. The *T. gondii* B1 gene was not detected in all residents examined. However, ELISA showed 8.0% (89 of 1,114 sera) positive for IgG antibodies against *T. gondii* in Seoul and 11.3% (117 of 1,036 sera) in Jeju-do. In both districts, the positive rates were higher in males than in females, and those 40-79 years of age showed higher rates than other ages. In Seoul, residents older than 70 years of age showed the highest positive rate, 14.9%, whereas in Jeju-do the highest prevalence, 15.6%, was in those in their sixties. The higher seropositive rate in Jeju-do than in Seoul may be related to eating habits and occupations. The present results and a review of related literature are indicative of an increased seroprevalence of *T. gondii* in Korea in recent years.

Key words: *Toxoplasma gondii*, seroprevalence, B1 gene, resident, Seoul, Jeju-do, IgG, ELISA

INTRODUCTION

Toxoplasma gondii, a coccidian protozoan, is distributed worldwide. This intracellular protozoan can infect essentially all nucleated eukaryotic cells of warm-blooded animals, including humans and vertebrate animals, and can cause clinical manifestations, such as lymphadenitis, encephalitis, retinochoroiditis, congenital infection, and neonatal mortality [1-3]. Transmission to humans occurs through ingestion of *T. gondii* bradyzoites or tachyzoites contained in undercooked meat of infected animals or ingestion of oocysts originating from the feces of infected cats [1-3]. Transplacental infection from mother-to-fetus is also well known [4]. In immunocompetent individuals, *T. gondii* infection is usually asymptomatic or causes only

mild symptoms [1-3,5]. However, it can elicit severe clinical manifestations in immunocompromised conditions, including AIDS, transplantation, malignant disease, and pregnancy [5]. Congenital toxoplasmosis may cause stillbirth, abortion, or other serious damages to the fetus, such as meningoencephalitis and hydrocephalus [4,5].

In the Republic of Korea (hereafter referred to as Korea), the seroprevalence of toxoplasmosis has been studied by many researchers [6-23]. From 1960 until 1999, residents, children, and hospital patients in Korea usually showed relatively lower prevalences, ranging from 1.1-7.7%, compared to American and European locales [3,5,6-13]. However, from 2000-2009, the seroprevalence of toxoplasmosis showed a tendency of slight increase [14-20]. In particular, the prevalence on Jeju-do Island (hereafter referred to as Jeju-do) among an adult population was reported as 12.9% [14]. Thereafter, the reported seroprevalence in Korean residents have been, with the exception of one report [21], higher than this figure, ranging from 13.2-25.8% [22-24]. In this respect, it has been assumed that the seroprevalence of toxoplasmosis in Korea is presently in-

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creasing, presumably due to an increased consumption of domestic or imported pork, or other animal meat at risk of *T. gondii* infection [23].

The present study was conducted to determine the seroprevalence of *T. gondii* among people residing in 2 districts, Seoul and Jeju-do, which have contrasting epidemiologic characteristics related to the parasite life cycle and transmission. In addition, in order to assess the current status and trend of toxoplasmosis, the literature reporting the seroprevalence of toxoplasmosis among Korean people is briefly reviewed.

MATERIALS AND METHODS

Subjects and sample collection

We collected sera and blood from 2,150 residents (1,114 in Seoul and 1,036 in Jeju-do; 12-95 years of age) from March to September 2011, during health check-up in the Eastern Seoul, Western Seoul, and Jeju-do branches of the Korea Association of Health Promotion (KAHP). The sera and blood were stored at -80°C until analyzed. This study was approved under the regulations established by the Institutional Review Board Committees of Seoul National University Hospital (No. C-1101-064-348, 17 April 2011), and KAHP (No. 11-C-02, 22 March 2011). Informed consent was obtained from each individual.

Preparation of *T. gondii* lysate antigen

Tachyzoites of *T. gondii* (RH strain) were collected from the peritoneal cavity of 6 week old BALB/c mice that had been previously injected 3-4 days before. The tachyzoites were washed 3 times with PBS and purified by centrifugation over 40% Percoll (Sigma-Aldrich, St. Louis, Missouri, USA). The tachyzoites were sonicated 5 times on ice and centrifuged at 100,000 g for 1 hr. The supernatant was collected for use as the soluble antigen. The protein content was measured using a Nanodrop 2000 spectrometer (Thermo Scientific, Wilmington, Delaware, USA).

ELISA to measure IgG titers

ELISA was performed as previously described [11] with slight modifications. Briefly, 200 µl of *T. gondii* lysate antigen (TLA) (5 µg/ml) in 0.05 M carbonate-bicarbonate buffer (pH 9.6) was coated on each well of a 96-well microtiter plate (Costar, Cambridge, Massachusetts, USA), and the plate was incubated overnight at 4°C. After washing, each well was reacted with serum samples that were diluted 1:100 with 0.05% Tween 20. After incubation at 37°C for 1 hr, a 1:10,000 dilution of horseradish

peroxidase-conjugated goat anti-human IgG (Bethyl Laboratories, Montgomery, Texas, USA) was applied, and the plate was incubated at 37°C for 1 hr. After several washes, freshly prepared *o*-phenylenediamine dihydrochloride (Sigma-Aldrich) was added, and the reaction was stopped by adding 8 N H₂SO₄. The IgG antibody titers were determined at the optical density of 490 nm.

PCR detection of *T. gondii* B1 gene in blood

The blood samples (n = 1,036) from Jeju-do were examined for the presence of *T. gondii* B1 gene using PCR. The DNeasy blood and tissue kit (Qiagen, Hilden, Germany) was used for isolation of the genomic DNA of *T. gondii*. To amplify the 497 bp fragment of the gene, PCR was carried out using the following forward Toxo 497 (5'-AGCAAACAC-CGACGAACTCT-3') and reverse Toxo 497 (5'-CATGGTTTGCATTTTGTTG-3') primers. The smart 2X PCR Pre-mix (Solgent, Daejeon, Korea) was used with the following conditions: 94°C for 5 min, followed by 35 cycles of 94°C for 60 sec, 55°C for 55 sec, and 72°C for 60 sec. The conventional PCR products were analyzed by 1% agarose gel electrophoresis and stained with ethidium bromide.

Statistical analysis

To analyze the risk factors for toxoplasmosis, the chi-square test was applied. $P < 0.05$ was considered statistically significant.

RESULTS

The serum IgG positive rate for *T. gondii*, as analyzed by ELISA, was 8.0% (89 positives among 1,114 sera) in Seoul and 11.3% (117 positives among 1,036 sera) in Jeju-do (Tables 1, 2). The average seropositive rate was 9.6% among all 2,150 residents (Table 1). The overall seroprevalence was a little higher in Jeju-do than in Seoul, although this difference was not statistically significant ($P > 0.05$). By age group, however, the seroprevalence appeared to be consistently higher in Jeju-do than in Seoul through the age groups of 30-39, 40-49, 50-59, and 60-69 years (Fig. 1). In both districts, aged residents, particularly, those > 40 years of age displayed markedly higher seroprevalences than younger residents ($P < 0.05$) (Fig. 1). In Seoul, the highest positive rate, 14.9%, revealed in residents ≥ 70 years old, whereas in Jeju-do, the highest prevalence, 15.6% (Fig. 1) was evident in those in their 60s. There were also differences in the seropositive rate of male and female residents. Males usually showed higher seropositive rate than females in

Table 1. Seroprevalence^a of *Toxoplasma gondii* infection among residents in Seoul and Jeju-do

District	Male		Female		Total	
	No. examined	No. positive (%)	No. examined	No. positive (%)	No. examined	No. positive (%)
Seoul	426	43 (10.1) ^b	688	46 (6.7) ^b	1,114	89 (8.0) ^d
Jeju-do	479	65 (13.6) ^c	557	52 (9.3) ^c	1,036	117 (11.3) ^d
Total	905	108 (11.9)	1,245	98 (7.9)	2,150	206 (9.6)

^aSerum samples were collected and examined during March-September 2011.
^{b,c,d}No statistically significant differences were found between the paired 2 figures ($P > 0.05$).

Table 2. Seroprevalence of *T. gondii* infection according to the occupation of subjects in Seoul and Jeju-do

Type of occupation	Seoul		Jeju-do		Total	
	No. examined	No. positive (%)	No. examined	No. positive (%)	No. examined	No. positive (%)
Agriculture	7	0 (0.0)	165	30 (18.2) ^a	172	30 (17.4)
Livestock	1	0 (0.0)	6	0 (0.0)	7	0 (0.0)
Fisheries	0	0 (0.0)	8	1 (12.5)	8	1 (12.5)
Office ^b	207	12 (5.8)	126	8 (6.3)	333	20 (6.0)
Housewife ^c	106	7 (6.6)	76	3 (3.9)	182	10 (5.5)
Forestry	6	0 (0.0)	2	1 (50.0)	8	1 (12.5)
Mining	2	1 (50.0)	2	0 (0.0)	4	1 (25.0)
Services	7	1 (7.1)	35	3 (8.6)	42	4 (9.5)
Miscellaneous	778	68 (8.7)	616	71 (11.5)	1,394	139 (10.0)
Total	1,114	89 (8.0)	1,036	117 (11.3)	2,150	206 (9.6)

^aResidents who are engaged in agriculture in Jeju-do showed higher seroprevalence than the average value, although the difference was not statistically significant ($P > 0.05$).
^{b,c}Office workers and housewives in Seoul or Jeju-do showed lower seroprevalence than the average value; however, the difference was not statistically significant ($P > 0.05$).

Seoul and Jeju-do (Table 1; Fig. 1). Particularly in Jeju-do, males showed higher seroprevalence than females with the exception of the age groups 20-29 year old and 30-39 year old age groups (Fig. 1). Overall, however, the sex difference of the seroprevalence was not statistically significant ($P > 0.05$) (Table 1).

The results of the questionnaire study on epidemiologic factors related with *T. gondii* infection showed several positive findings, although statistical significance was not confirmed for all of the positive factors (Tables 2, 3). With regard to occupation, the 18.2% seropositive rate among Jeju-do residents who engaged in agriculture was markedly higher than the average value of 11.3%, although there was no statistical significance ($P > 0.05$) (Table 2). Similarly, officers and housewives in Seoul and Jeju-do exhibited slightly lower seroprevalences (5.8-6.6% and 3.9-6.3%, respectively) than the average values (8.0% and 11.3%, respectively). Again, there was no statistical significance also ($P > 0.05$) (Table 2).

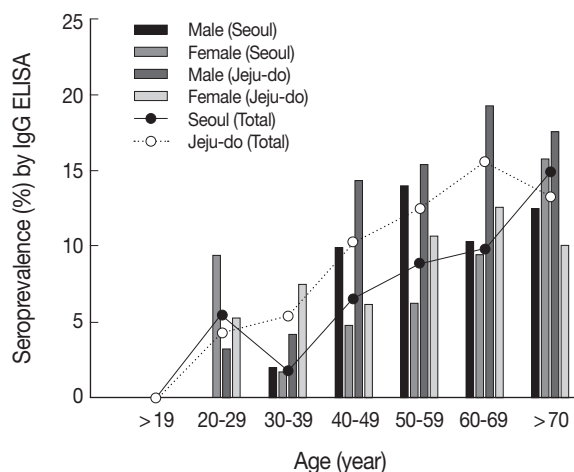


Fig. 1. Age- and sex-specific seroprevalence of toxoplasmosis among residents in Seoul and Jeju-do assayed by IgG ELISA. The seroprevalence was generally higher in Jeju-do than in Seoul and in males than in females both in Seoul and Jeju-do, although these differences were not statistically significant ($P > 0.05$).

The residents who preferred to eat a vegetarian diet were less likely to have infection with *T. gondii* (Table 3). The role of this factor was statistically significant particularly in Seoul ($P < 0.05$). Jeju-do residents who drank bottled water were significantly less likely to get infections ($P < 0.05$) (Table 3). Other factors, including the history of contact with animals and travel abroad, did not appear to be related with *T. gondii* infection both in Seoul and Jeju-do (Table 3).

The B1 gene of *T. gondii* was negative in all of the 1,036 blood samples collected from Jeju-do (samples from Seoul were not examined for the B1 gene), whereas the positive control containing *T. gondii* RH tachyzoites exhibited positive reaction band in PCR (data not shown).

DISCUSSION

The latex agglutination test (LAT) has been one of the most reliable diagnostic methods for toxoplasmosis for almost 4

Table 3. Relationship of epidemiologic factors and serologic results of *T. gondii* infection among residents in Seoul and Jeju-do

Epidemiologic factor	Seoul		Jeju-do		Total	
	No. seropositive (T ^a)	No. sero-negative	No. seropositive (T ^a)	No. sero-negative	No. seropositive (T ^a)	No. sero-negative
Vegetarian diet	11 ^b (21)	255	12 (16)	136	23 ^b (40)	391
No	78 ^b (67)	770	105 (100)	783	183 ^b (166)	1,553
Using bottled water	49 (47)	551	44 ^b (56)	457	93 (105)	1,008
No	40 (41)	474	73 ^b (60)	462	113 (101)	936
Cooked food intake	80 (81)	942	83 (87)	688	163 (172)	1,630
No	9 (7)	83	34 (29)	231	43 (34)	314
Contact with animals	15 (12)	144	28 (21)	166	43 (34)	310
No	74 (76)	881	89 (95)	753	163 (172)	1,634
History of travel abroad	24 (28)	333	50 (54)	430	74 (80)	763
No	65 (60)	692	67 (62)	489	132 (126)	1,181

^aTheoretical values for the observed ones (no. of seropositive cases).

^bStatistical significance was noted ($P < 0.05$) between these 2 variables.

decades [7,8,20]. However, its sensitivity has been estimated to be lower than that by ELISA both for serum and cerebrospinal fluid [11]. Therefore, nowadays in Korea, ELISA is more popularly used than LAT to estimate the seroprevalence of toxoplasmosis [12-19,21-24]. ELISA can accurately measure IgG, IgM, IgA, or IgE antibodies [25]. Detection of IgG antibody implies a chronic latent infection, whereas detection of IgM or IgA antibody is indicative of an acute infection [21,25]. However, IgM ELISA has a risk of false-positive results due to anti-nuclear antibodies or rheumatoid factor, or of detection of residual or persistent IgM months or even years after the primary infection [25]. Alternatively, IgG avidity test is performed to detect acute cases [25-27], and PCR for the *T. gondii* B1 gene is done to detect congenital or chronic clinical cases [28,29].

In the present study, IgG ELISA and PCR for the *T. gondii* B1 gene were carried out. All of the PCR-tested blood samples from Jeju-do revealed negative results. This may indicate that there were no acute clinical toxoplasmosis cases among the subjects examined. Unless a subject is suffering from acute infection, it is less likely for the *T. gondii* B1 gene to be detected from blood. Meanwhile, in IgG ELISA, 8.0% of residents in Seoul and 11.3% in Jeju-do revealed positive results. The IgG positive rates were higher in males than in females, and those from the forties to the seventies were more likely to be infected than younger individuals.

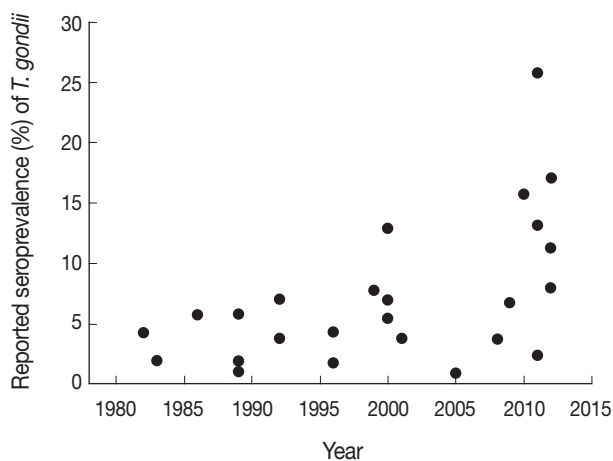
In the questionnaire, residents who preferred to eat a vegetarian diet and to drink bottled water were revealed to have a significantly lower ($P < 0.05$) seroprevalence than those who ate meat and who consumed groundwater or well water. Those engaged in agriculture had a higher rate of seropositivity, whereas office workers and housewives tended to show lower sero-

prevalence, although there was no statistical significance ($P < 0.05$) related with these occupation factors. It seems highly plausible that meat consumption is more closely related to infection with *T. gondii* than other possible factors in these 2 districts of Korea. Similarly, the higher seropositive rate in Jeju-do than in Seoul seems to be related to eating habits. It should be ruled out that the significantly lower seropositive rate among those who drank bottled water than those who consumed ground-water or well water might have been due to a lower risk of infection with *T. gondii* oocysts through bottled water.

Compared with previous studies conducted in various localities from 1960 until 2009, the results of the present study demonstrated fairly high seroprevalence of *T. gondii* infection among residents of Seoul and Jeju-do (Table 4; Fig. 2). For example, in Seoul, 1.1-7.0% seroprevalence was reported in 1982-1992 [7-11]. Thereafter, among children under 10 years of age in Seoul, a 1997 study revealed 7.7% seropositivity [13]. However, pregnant women in Seoul showed a prevalence of only 0.9% in 2005 [18]. Therefore, the prevalence of 8.0% in the present study is the highest seroprevalence of toxoplasmosis ever reported from Seoul. With regard to Jeju-do, the seroprevalence was first studied in 1989 and 5.8% seroprevalence was reported from patients in a hospital [10]. Since then, several studies have reported the seroprevalence of toxoplasmosis in Jeju-do. A survey of high school students and adult hospital patients in Jeju-do revealed 5.5% and 12.9% seroprevalence, respectively [14]. In 2011 examination of outpatients of a general hospital in Jeju-do revealed a 13.2% IgG ELISA positive rate [22]. Compared with these reported seroprevalences, the 11.3% seroprevalence observed in the present study appears to indicate a sustained, rather than decreased, level in Jeju-do.

Table 4. Comparison of *Toxoplasma gondii* seropositive rates in Korea reported by different authors

Authors (year)	Subjects	Surveyed area	No. positive/ No. examined	Seropositive rate (%)	Assay method	Reference
Soh et al. (1960)	Residents	Korea	21/373	5.8	Skin test	[6]
Choi et al. (1982)	Patients in a university hospital	Seoul	18/421	4.3	LA ^a	[7]
Choi et al. (1983)	Mental patients	Seoul	11/573	1.9	LA	[8]
Yoo and Choe (1986)	Children (< 15 years)	Seoul	7/120	5.8	Hemag-glutination	[9]
Choi et al. (1989)	Patients in a hospital	Seoul	19/1,019	1.9	LA	[10]
	Asthma patients	Seoul	11/1,030	1.1	LA	
	Patients in a hospital	Jeju-do	45/780	5.8	LA	
Choi et al. (1992)	Neurologic patients	Seoul	76/2,016	3.8	LA	[11]
	Neurologic patients	Seoul	142/2,016	7.0	ELISA	
Ryu et al. (1996)	Pregnant women	Gyeonggi-do	39/899	4.3	ELISA	[12]
	High school students	Gyeonggi-do	4/218	1.8	ELISA	
Kook et al. (1999)	Under 10-year children	Seoul	42/542	7.7	LA	[13]
Yang et al. (2000)	Students	Jeju-do	250/4,570	5.5	ELISA	[14]
	Adults	Jeju-do	61/474	12.9	ELISA	
Hong et al. (2000)	High school students	Jeju-do	250/4,320	5.5	ELISA	[15]
Lee et al. (2000)	Residents	Okcheon-gun	74/1,109	6.9	ELISA	[16]
Bae et al. (2001)	Teachers of childbearing age	Jeju-do	12/314	3.8	LA/ELISA	[17]
Song et al. (2005)	Pregnant women	Seoul	51/5,725	0.9	ELISA	[18]
Han et al. (2008)	Pregnant women	Daejeon	13/351	3.7	ELISA/LA	[19]
Shin et al. (2009)	Patients in a university hospital	Daejeon	85/1,265	6.6	LA	[20]
			84/1,265	6.7	ELISA	
Ko et al. (2011)	Pregnant women	Daejeon	19/787	2.4	ELISA	[21]
Hong et al. (2011)	Residents	Jeju-do	309/2,348	13.2	ELISA	[22]
Yang et al. (2012)	Residents (2010)	Gyeonggi-do	195/1,232	15.8	ELISA	[23]
	Residents (2011)	Gyeonggi-do	335/1,296	25.8	ELISA	
Ahn et al. (2012)	Residents	Gangwon-do	282/1,661	17.0	ELISA	[24]
Lim et al. (2012) ^b	Residents	Seoul	89/1,114	8.0	ELISA	-
	Residents	Jeju-do	117/1,036	11.3	ELISA	-

^aLA; latex agglutination test.^bPresent study.**Fig. 2.** Chronologic view of the seroprevalence of toxoplasmosis reported in Korea (1982-2012) based on data in Table 4 [7-24]. Each point represents % seropositive rate for each subjected group surveyed. Note that the seroprevalence in recent years (2010-2012) showed an increasing tendency.

In recent years, an increasing tendency in the seroprevalence of toxoplasmosis in Korea is evidently recognizable (Table 4; Fig. 2). Particularly, on islands in Gangwon-gun, the seroprevalence among residents were 15.8% in 2010 and 25.8% in 2011 which was the highest figure ever reported [23]. Subsequently, in Cheorwon-gun, the IgG ELISA seropositive rate was also high, at 17.0% [24]. The reason for this increasing tendency of seroprevalence is unclear, although an increase in the number of stray cats [30] and more popular consumption of meat, including pork, by Koreans [31] can be suggested as the possible reasons. Nevertheless, it should also be ruled out that the serologic techniques to detect IgG antibodies have been much improved and became more sensitive compared to those used in previous studies. This issue should be clarified in the near future.

The results of the present study strongly suggest that public health attention should be paid to toxoplasmosis prevalence in Korea.

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