



High Seroprevalence of Toxoplasmosis Detected by RDT among the Residents of Seokmo-do (Island) in Ganghwa-Gun, Incheon City, Korea

Yeong Hoon Kim¹, Jihoo Lee², Seongkyu Ahn³, Tong-Soo Kim³, Sung-Jong Hong⁴, Chom-Kyu Chong², Hye-Jin Ahn⁵, Ho-Woo Nam^{5,*}

¹Department of Ophthalmology, College of Medicine, Catholic University of Korea, Seoul 06591, Korea; ²Genbody Inc., Cheonan 31116, Korea; ³Department of Parasitology and Tropical Medicine and Inha Research Institute for Medical Sciences, Inha University School of Medicine, Incheon 22333, Korea; ⁴Department of Medical Environmental Biology, Chung-Ang University College of Medicine, Seoul 06974, Korea; ⁵Department of Parasitology, College of Medicine, the Catholic University of Korea, Seoul 06591, Korea

Abstract: Seroprevalence of *Toxoplasma gondii* infection among the residents of Seokmo-do (Island) in Ganghwa-gun, Incheon, Korea was surveyed for 4 years by a rapid diagnostic test (RDT) using recombinant fragment of major surface antigen (SAG1), GST-linker-SAG1A. Sera from 312, 343, 390, and 362 adult residents were collected on a yearly basis from 2010 to 2013, respectively. Total positive seroprevalence regardless of gender was 29.2, 35.3, 38.7, and 45.3% from 2010 to 2013, respectively. Positive seroprevalence in male adults was 43.9, 48.2, 45.4, and 55.3%, which was far higher than that of the corresponding female adults which was 20.7, 29.2, 33.9, and 38.9%, from 2010 to 2013, respectively. This high seroprevalence of toxoplasmosis in Seokmo-do may have been caused in part by peculiar changes in the toxoplasmic environment of the island as it is a relatively isolated area preserving its natural habitat while also being connected by a bridge to the mainland. Further study is necessary to find out symptomatic patients and to confirm the risk factors.

Key words: *Toxoplasma gondii*, seroprevalence, rapid diagnostic test, Seokmo-do (Island), Korea

INTRODUCTION

Toxoplasma gondii, an apicomplexan zoonotic protozoa, is one of the most successful parasites widely prevalent in humans and animals worldwide. Felids are its definitive host, and almost all other warm blooded animals, including humans, are its intermediate hosts. Toxoplasmosis is acquired by the infection of *T. gondii* through ingestion of tissue cysts from undercooked meat, or consuming food or water contaminated with oocysts. Another route of infection is from infected mother to her fetus via placental transmission, a process known as congenital toxoplasmosis [1-3]. Congenital toxoplasmosis may cause stillbirth or abortion in addition to serious damages to the fetus, such as severe neurological disorders after delivery [4,5]. Most toxoplasmosis cases show only mild

symptoms or are asymptomatic as only a small percentage of exposed adult humans develop clinical symptoms following exposure. The infections can transform into a chronic status, especially in the central nervous system of the individuals affected. The infection is sometimes reactivated in immune compromised patients to cause toxoplasmic lymphadenitis, meningoencephalitis, or ocular toxoplasmosis [6-9].

Many surveys on the seroprevalence of toxoplasmosis among various patient groups have been performed in Korea using various test methods [10-15]. The seroprevalence resulted in the range from 1.9 to 5.8% with indirect latex agglutination (ILA) test [10] and up to 7.7% with the more sensitive ELISA [11]. On the other hand, all of these were still significantly lower than those of the other countries with seroprevalences of 30-70% [3]. However, recent surveys have demonstrated higher seroprevalences ranging from 12.9 to 17.0% in certain parts of Korea [10,12-14]. Although ELISA has been used widely for diagnosis of toxoplasmosis, it is a time-consuming procedure and requires a skilled technician. Thus, it is being replaced gradually by a rapid diagnostic test (RDT), the current universal trend for diagnosis.

•Received 9 November 2016, revised 9 January 2017, accepted 30 January 2017.

*Corresponding author (howoo@catholic.ac.kr)

© 2017, Korean Society for Parasitology and Tropical Medicine

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Korea has an almost 100% full health-coverage of its population, yet its seroprevalence of toxoplasmosis in certain areas is high or surging recently, which suggests some changes in the toxoplasmic environment [14]. The current study presents 4-year-results of a survey on the seroprevalence of toxoplasmosis performed by RDT among the residents in Seokmo-do (Island) in Ganghwa-gun, Incheon City, localized in the Yellow Sea. We tried to postulate its significance and also the cause of high prevalence of toxoplasmosis in this area.

MATERIALS AND METHODS

Ethics statement

This study was performed under the regulation of the IRB Committee of Chung-Ang University (no. 2010-06-03) and the IRB Committee of Inha University (nos. 2012-28 and 2013-008). This research adhered to the tenets of the Declaration of Helsinki. All participants provided written informed consent, and no minors were involved in the study.

All procedures and handling of mice were conducted under an approved protocol by the Institutional Animal Care and Use Committee (IACUC) at the School of Medicine, Catholic University of Korea (no. CUMC-2015-0042-02, 2015-2017), which adhered to the regulations set under the Korean National Animal Protection Act. Tachyzoites of *T. gondii* (RH strain) were provided by the National Veterinary Research and Quarantine Service [16].



Fig. 1. The surveyed area. Administratively Seokmo-do (Island) belongs to Samsan-myeon, and Samsan-myeon is a part of Ganghwa-gun, Incheon City, in the western part of Korea.

Parasite and preparation of antigen

T. gondii tachyzoites (RH) were maintained by peritoneal passages in BALB/c mice. The tachyzoites were purified by centrifugation over 40% Percoll (Sigma-Aldrich, St. Louis, Missouri, USA) in PBS [17] and disrupted by sonification in a digital sonifier (Branson, Danbury, Connecticut, USA). The concentration of protein was measured with a Bio-Rad protein assay kit (Bio-Rad, Hercules, California, USA).

Serum collection

Sera from 312, 343, 390, and 362 adult residents in Seokmo-do, Ganghwa-gun, Incheon, Korea (Fig. 1) were collected on a yearly basis from 2010 to 2013 (Table 1).

Rapid diagnostic test (RDT)

IgG/IgM RDT mounted with recombinant fragment of major surface antigen (SAG1A), GST-linker-SAG1A [12,18], were applied to the sera mentioned above. Briefly, 10 μ l of serum was applied to the RDT sample hole and eluted with RDT buffer a few seconds later. Reacting bands were read by 3 investigators after 15-20 min, and its density was determined arbitrarily as 0, +, ++, and +++. Definite reactivity was determined as ++ (mid), while weaker and stronger reactivity compared to it was determined as + (weak) and +++ (strong). The final results were agreed upon by the 3 investigators.

RESULTS

The positive seroprevalence of the residents in Seokmo-do was high from the beginning of the survey in 2010 at 29.2% (91/312). The seroprevalence was maintained high in subsequent years, and simultaneously showed an increasing trend, to 35.3% (121/343), 38.7% (151/390), and 45.3% (164/362) in 2011, 2012, and 2013, respectively (Table 1; Fig. 2).

According to gender, the positive rate of males was always higher than that of females, maintaining roughly a 2-fold

Table 1. Seroprevalence of *T. gondii* by RDT among the residents of Seokmo-do (Island) in Ganghwa-gun, Incheon City, Korea, 2010-2013, according to gender

Year	Total	Male	Female
2010	91/312 (29.2) ^a	50/114 (43.9)	41/198 (20.7)
2011	121/343 (35.3)	53/110 (48.2)	68/233 (29.2)
2012	151/390 (38.7)	74/163 (45.4)	77/227 (33.9)
2013	164/362 (45.3)	78/141 (55.3)	86/221 (38.9)

^aNo. positive/No. examined (%).

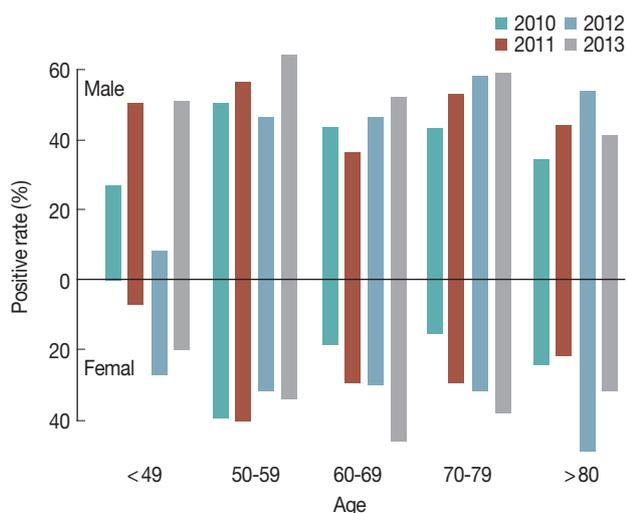


Fig. 2. Trends in positive seroprevalence according to gender and age in the surveyed area.

higher ratio throughout the survey period. On the other hand, the positive rate of females showed a steeper increase, while males showed a more gradual increase. In regard to age, positive rates increased gradually in all age groups, regardless of gender. However, a sharp drop was observed in the < 49 years-old male group in 2012. Additionally, during the early stages of the survey (2010 and 2011), the positive rates were fairly low in the < 49 years-old female group.

When comparing the age groups according to gender, the positive rate of men was always higher than that of women in the same age group, as noted above. Nevertheless, positive seroprevalence showed a steady increasing trend in both genders (Fig. 2). RDT reactivity changes were also observed in seropositive cases during the survey period. The ratio of the mid-reactivity group was relatively constant. However, the strong-reactivity group showed a gradual increase in its ratio, while the weak-reactivity group showed a gradual decrease (Fig. 3).

DISCUSSION

Since Soh et al. [19] first reported the seropositive rate of 5.6% in 373 Koreans by skin test using toxoplasmin in 1960, several serological methods such as indirect fluorescent antibody test (IFAT), ILA, and ELISA have been developed for the detection of *T. gondii* infection. However, most of them are expensive and time-consuming, and recently RDT using recombinant proteins as antigens has been developed to address these problems [18].

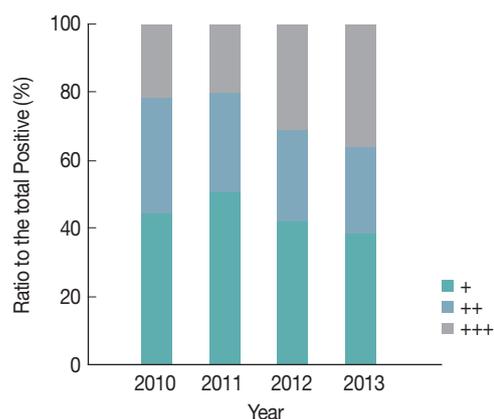


Fig. 3. Changes of RDT reactivity in seropositive cases.

The seroprevalence of the residents in Seokmo-do was high from the beginning of the survey in 2010 at 29.2% (91/312). The seroprevalence was maintained high in subsequent years, and simultaneously showed an increasing trend. This high seroprevalence may have been caused in part by peculiar changes in the toxoplasmic environment of the island as it is a relatively isolated area preserving its natural habitat while also being connected by a bridge to the mainland.

Meat consumption is increasing in Korea, but domestic meat supply is limited, so a large amount must be imported. As the world becomes more global day by day, the meat source also becomes more varied. Some of them may have been imported from high endemic nations. Indeed, pork has been imported without any inspection on *T. gondii* infection in Korea [15].

Ganghwa-gun is known as an outbreak site of toxoplasmic lymphadenitis by the ingestion of tissue-cyst from pork which was fed in a backyard pigsty. Regardless of pig farm or house pigsty, pigs have been bred under conditions where cats can access easily. They are served as popular foods in domestic festivals, wedding receptions and funeral receptions, and local markets. This may presumably explain the high positive rates observed on the area, if pork had been served undercooked. These factors, combined with its isolated location may have provided a high parasite burden to the inhabitants of the island.

Like most other advanced countries, Korea has seen an alarming increase in the elderly population recently. People in rural areas have traditionally kept many pets, but the increase in single households and life expectancy has caused an explosive increase in these pets. This again has resulted in an increase in abandoning them, as most of them cannot be cared

when their owners pass away. Most of them become feral and may contribute to the increase in toxoplasmosis prevalence. Interaction among pets and these feral counterparts are also common in the countryside. Especially troublesome is an increase in the number of feral cats, they became ubiquitous in Korea, and have occupied the top of the food chain in the Korean fauna, with increasing oocyst-shedding rate. Additionally, as mentioned above, Seokmo-do is connected to the mainland by a bridge, so access for feral cats is far easier than the past.

Korea is becoming economically ever more affluent, and health consciousness is increasing, along with environmental awareness. Hence, there is an increase in organic agriculture with far less use of pesticides throughout the country [20,21]. On the other hand, the improvement in the environment has caused an increase in the local fauna, and thus the potential for intermediate *Toxoplasma* hosts have also increased [22]. Sometimes they are captured by a snare to be consumed among families and relatives.

Another significant source of infection, albeit minor, could be migratory birds [23,24] as Ganghwa-gun has been renowned for its extensive tideland marshes, an important shelter for these birds. During the recent avian influenza crisis, a high number of migratory birds have been positively identified with the virus, so these birds may also transmit toxoplasmosis from high endemic regions [25].

The positive seroprevalence of men was always higher than that of women, maintaining roughly a 2-fold higher ratio throughout the survey period. Some Koreans, mostly men, still have the misbelief that the raw viscera of animals, both domestic and wild, have special nutrients for their stamina, so they eat them raw. This may in some part explain the higher *Toxoplasma* seroprevalence in men observed in our study, besides being more socioeconomically active. On the other hand, the positive rate of women showed a steeper increase, while males showed a more gradual increase. Recent increase in consumption of imported pork, and more intimate relationship between elderly women and their pets may have been partially responsible. Further study is deemed necessary to address this problem.

In regard to age, positive rates increased gradually in all age groups, regardless of gender, but a sharp drop was observed in <49 years-old male group (2012), and they were absent or fairly low in <49 years-old female group. These may reflect the demographic changes in rural areas where young generations leave for cities for better opportunities.

A peculiarity of RDT needs to be addressed in regard to our study. Our previous study has determined the overall specificity and sensitivity of RDT to be 100% and 97.1%, respectively [18]. RDT uses rSAG1A-GRA2 antigen. A chronic infection history implies abundant antibodies against various antigens, such as SAGs, GRAs, and MICs, which could be detected by ELISA, whereas RDT would be limited in this respect, as only 2 antigens are detected. RDT reactivity changes were also observed in seropositive cases during the survey period. The ratio of the mid-reactivity group was relatively constant, but the strong-reactivity group showed a gradual increase in its ratio, while the weak-reactivity group showed a gradual decrease. Taking these factors together, it may be implied that the high positive seroprevalence indicates an increasing incidence of new cases, which would be better detected by RDT. These cases may represent an early or acute toxoplasmosis.

Toxoplasmosis is prevalent throughout the world, in both humans and our environment, but clinical manifestations are somewhat uncommon, so it is being mainly neglected in major health policies. However, our study reveals a different story, and that a worrying one. A major surge in seroprevalence, coupled with a change in disease characteristics at any point, may cause a major health threat.

In conclusion, it is necessary to find both symptomatic and asymptomatic toxoplasmic patients and confirm the risk factors for further infection in the area. Repeated surveys at a certain time interval are recommended to monitor the infection status, including new infections. Indeed, the problems may not be limited to the island, so the postulated causes in this study may be applied to a full-scale national survey to screen the national toxoplasmic seroprevalence.

ACKNOWLEDGMENT

This study was supported by a research grant from the Korea Association of Health Promotion (no. 2015-01), Republic of Korea.

CONFLICT OF INTEREST

We have no conflict of interest related to this work.

REFERENCES

1. Black MW, JC Boothroyd. Lytic cycle of *Toxoplasma gondii*. Micro-

- biol Mol Biol Rev 2000; 64: 607-623.
2. Furtado JM, Smith JR, Belfort, R Jr., Gattety D, Winthrop KL. Toxoplasmosis: a global threat. *J Glob Infect Dis* 2011; 3: 281-284.
 3. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol* 2000; 30: 1217-1258.
 4. Montoya JG, Remington JS. Management of *Toxoplasma gondii* infection during pregnancy. *Clin Infect Dis* 2008; 47: 554-566.
 5. Song KJ, Shin JC, Shin HJ, Nam HW. Seroprevalence of toxoplasmosis in Korean pregnant women. *Korean J Parasitol* 2005; 43: 69-71.
 6. Weiss LM, Dubey JP. Toxoplasmosis: A history of clinical observations. *Int J Parasitol* 2009; 39: 895-901.
 7. Choi WY, Nam HW, Kwak NH, Huh W, Kim YR, Kang MW, Cho SY, Dubey JP. Foodborne outbreaks of human toxoplasmosis. *J Infect Dis* 1997; 175: 1280-1282.
 8. Kim MH, Choi YK, Park YK, Nam HW. A toxoplasmic uveitis case of a 60-year-old male in Korea. *Korean J Parasitol* 2000; 38: 29-31.
 9. Park YH, Han JH, Nam HW. Clinical features of ocular toxoplasmosis in Korean patients. *Korean J Parasitol* 2011; 49: 167-171.
 10. Choi WY, Nam HW, Youn JH, Kim WS, Kim WK. *Toxoplasma* antibody titers by indirect latex agglutination test in patients of Kangnam St. Mary's Hospital and Cheju Medical Center. *Korean J Parasitol* 1989; 27: 171-175.
 11. Choi WY, Nam HW, Youn JH, Kim DJ, Kong Y, Kang SY, Cho SY. Detection of antibodies in serum and cerebrospinal fluid to *Toxoplasma gondii* by indirect latex agglutination test and enzyme-linked immunosorbent assay. *Korean J Parasitol* 1992; 30: 83-90.
 12. Hong SJ, Chong CK, Lee K, Kim TS, Hong YP, Ahn HJ, Kim HY, Ko AR, Kim YJ, Nam HW. Maintained seroprevalence of toxoplasmosis among the residents of Jeju island, Korea. *Korean J Parasitol* 2011; 49: 309-311.
 13. Yang HJ, Jin KN, Park YK, Hong SC, Bae JM, Lee SH, Choi HS, Hwang HS, Chung YB, Lee NS, Nam HW. Seroprevalence of toxoplasmosis in the residents of Cheju island, Korea. *Korean J Parasitol* 2000; 38: 91-93.
 14. Ahn HJ, Cho PY, Ahn SK, Kim TS, Chong CK, Hong SJ, Cha SH, Nam HW. Seroprevalence of toxoplasmosis in the residents of Cheorwon-gun, Gangwon-do, Korea. *Korean J Parasitol* 2012; 50: 225-227.
 15. Yang Z, Cho PY, Ahn SK, Ahn HJ, Kim TS, Chong CK, Hong SJ, Cha SH, Nam HW. A surge in the seroprevalence of toxoplasmosis among the residents of islands in Ganghwa-gun, Incheon, Korea. *Korean J Parasitol* 2012; 50: 191-197.
 16. Choi WY, Nam HW, Yoo JE. Toxoplasma-cidal effect of HL-60 cells differentiated by dimethylsulfoxide. *Korean J Parasitol* 1988; 26: 229-238.
 17. Sohn WM, Nam HW. Western blot analysis of stray cat sera against *Toxoplasma gondii* and the diagnostic availability of monoclonal antibodies in sandwich-ELISA. *Korean J Parasitol* 1999; 37: 249-256.
 18. Song KJ, Yang Z, Chong CK, Kim JS, Lee KC, Kim TS, Nam HW. A rapid diagnostic test for toxoplasmosis using recombinant antigenic N-terminal half of SAG1 linked with intrinsically unstructured domain of gra2 protein. *Korean J Parasitol* 2013; 51: 503-510.
 19. Soh CT, Lee SJ, Ahn YK. Latent infection by *Toxoplasma gondii* in Korea. *Yonsei Med J* 1960; 1: 52-54.
 20. Dubey JP, Hill DE, Rozeboom DW, Rajendran C, Choudhary S, Ferreira LR, Kwok OC, Su C. High prevalence and genotypes of *Toxoplasma gondii* isolated from organic pigs in northern USA. *Vet Parasitol* 2012; 188: 14-18.
 21. Meerburg BG, De Craeye S, Dierick K, Kijlstra A. *Neospora caninum* and *Toxoplasma gondii* in brain tissue of feral rodents and insectivores caught on farms in the Netherlands. *Vet Parasitol* 2012; 184: 317-320.
 22. Kim DG, Park JH, Kim JL, Jung BK, Jeon SJ, Lim H, Lee MY, Shin EH, Klein TA, Kim HC, Chong ST, Song JW, Baek LJ, Chai JY. Intestinal nematodes from small mammals captured near the demilitarized zone, Gyeonggi province, Republic of Korea. *Korean J Parasitol* 2015; 53: 135-139.
 23. Verma SK, Calero-Bernal R, Cerqueira-Cézar CK, Kwok OC, Dudley M, Jiang T, Su C, Hill D, Dubey JP. Toxoplasmosis in geese and detection of two new atypical *Toxoplasma gondii* strains from naturally infected Canada geese (*Branta canadensis*). *Parasitol Res* 2016; 115: 1767-1772.
 24. Sandström CA, Buma AG, Hoye BJ, Prop J, van der Jeugd H, Volslamber B, Madsen J, Loonen MJ. Latitudinal variability in the seroprevalence of antibodies against *Toxoplasma gondii* in non-migrant and arctic migratory geese. *Vet Parasitol* 2013; 194: 9-15.
 25. Hill NJ, Ma EJ, Meixell BW, Lindberg MS, Boyce WM, Runstadler JA. Transmission of influenza reflects seasonality of wild birds across the annual cycle. *Ecol Lett* 2016; 19: 915-925.