

Seroprevalence of Toxoplasmosis with ELISA and Rapid Diagnostic Test among Residents in Gyodong-do, Incheon city, Korea: A Four-Year Follow-up

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Abstract: ELISA has been used for the diagnosis of toxoplasmosis, but it is being gradually replaced by a rapid diagnostic test (RDT). We compared and analyzed ELISA and RDT results using the sera collected during 4 consecutive years from residents of Gyodong-do (Island), Incheon-city, Korea. Sera from 921, 993, 940, and 838 adult residents were collected on a yearly basis (2010-2013). ELISA was performed by using a crude extract of *T. gondii* RH strain antigen and IgG/IgM RDT mounted with recombinant fragment of major surface antigen (SAG1), GST-linker-SAG1A, were applied to the sera. Comparison between groups was analyzed by the Student's t-test. The positive seroprevalence surged from 14.7% (135/921, 2010), 23.1% (231/993, 2011), 23.6% (222/940, 2012), and 32.1% (269/838, 2013) by ELISA. In contrast, RDT showed a more moderate increasing trend from 21.7% (200/921, 2010), 25.5% (253/993, 2011), 28.9% (272/940, 2012) and 33.1% (277/838, 2013). Discrepancies between ELISA and RDT were noted near the cut-off value. At the OD 0.15-0.24 range, RDT could detect 16.1% (169/1051) more positives, which suggests an early or acute toxoplasmosis, but at the OD 0.25-0.34 range, ELISA could detect 35.9% (92/256) more positives of possible chronic infections. Over the OD > 0.35 ELISA and RDT agreed in the majority of the cases. This surge in seroprevalence may be caused by the organic agriculture in addition to eating behavior or increase in pets among Koreans. These facts may be applied on a full-scale national survey using RDT to supplement ELISA to define the characteristics of the infection.

Key words: *Toxoplasma gondii*, seroprevalence, Gyodong-do (Island), RDT, ELISA

INTRODUCTION

Toxoplasma gondii, an apicomplexan zoonotic protozoan, is one of the most successful parasites widely prevalent in humans and animals worldwide. Felids are its definitive host, and all the other warm blooded animals, including humans, are its intermediate hosts. Toxoplasmosis is acquired by the infection with *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 of *T. gondii*, 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]. Only a small percentage of exposed adult humans develop clinical symptoms following exposure, so that most cases show only mild symptoms or are asymptomatic. However, the infections can change into a chronic status, especially in the central nervous system of the individuals affected. These sometimes reactivate in immune compromised patients to cause toxoplasmic lymphadenitis, meningoencephalitis, or ocular toxoplasmosis [6-9].

Many serological tests have been developed to test anti-*Toxoplasma* antibodies in Korea. Of these, indirect latex agglutination (ILA) test had been used for several years because of its easiness to perform and its comparable sensitivity and specificity to the dye test after improvements by Tsubota et al. [10] and Kobayashi et al [11]. There have been many surveys on the seroprevalence of toxoplasmosis among various groups of patients in Korea, which resulted in the range from 1.9 to 5.8%

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with ILA test [12] and up to 7.7% with the more sensitive ELISA [13], all of which are still significantly lower by approximately 1/10 than those of the other countries with a positive seroprevalence of 30 to 70% [3]. Recently, we have proved the maintained seroprevalence of toxoplasmosis in Jeju-do (Island) over 30 years [12,14,15], which is higher than the other regions of Korea at 12.9% to 13.2%. We also observed a very high seroprevalence rate of 17.0% in residents of Cheorwongun, located in mainland Korea, which suggests some changes in the toxoplasmic environment [16]. Although ELISA has been used widely in the diagnosis of toxoplasmosis, it is a time-consuming procedure, and there is a need for a skilled technician, which have resulted in its being replaced gradually by a rapid diagnostic test (RDT), the current universal trend for diagnosis.

Korea is on the verge of becoming an advanced country with an almost 100% full health-coverage of its population, yet its seroprevalence of toxoplasmosis in certain areas is surging recently. The surge is worrying, and one factor contributing to it may be climatic changes as the Korean peninsula is experiencing sub-tropical weathers during the warm periods of the year.

The current study compared and analyzed ELISA and RDT for the detection of anti-*Toxoplasma* antibodies with the sera of residents in Gyodong-do (Island), Incheon, Korea for 4 years from 2010 to 2013. An explosive increase in the seroprevalence

was noted in the area. The same results were observed both by ELISA and RDT, but discrepancies were present around the cut-off positive value of OD 0.25 in ELISA with RDT. We tried to postulate its significance and also the cause of the recent sudden surge 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 (no. 2012-28 and 2013-008). This research adhered to the tenets of the Declaration of Helsinki. All participants were provided with a 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. The RH strain of *T. gondii* has been provided by the National Veterinary Research and Quarantine Service, the Republic of Korea [17].

Parasite and preparation of antigen

The RH strain of *T. gondii* was maintained by peritoneal pas-

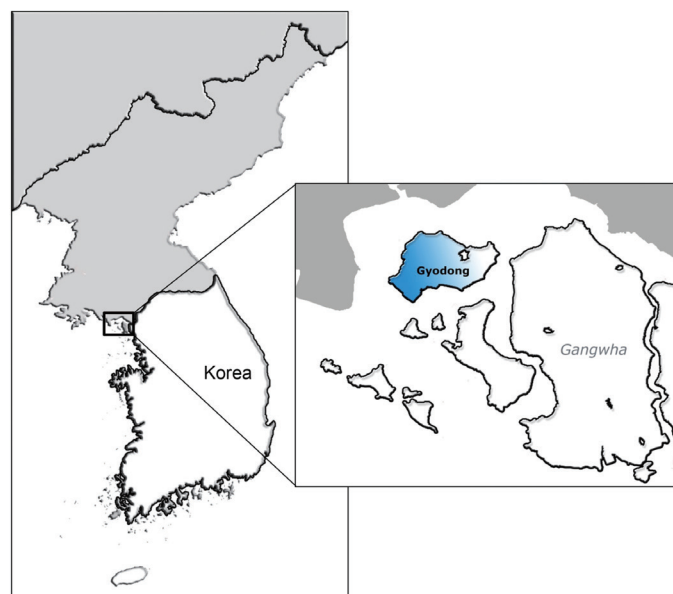


Fig. 1. The surveyed area. Gyodong-do (Island) administratively belongs to Gyodong-myeon, which is a part of Gangwha-gun, Incheon, in the western part of Korea.

sages in BALB/c mice. The tachyzoites were purified by centrifugation over 40% Percoll (Sigma-Aldrich Co., St. Louis, Missouri, USA) in PBS [18] 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 of 921, 993, 940, and 838 adult residents in Gyodong-do (Gyodong-myeon, Gangwha-gun, Incheon, Korea) (Fig. 1) were collected on a yearly basis from 2010 to 2013 (Table 1). Both ELISA and RDT were performed on the samples to counter-check each other.

ELISA

The sera were checked for IgG antibody titers by ELISA using a crude extract RH strain antigen of *T. gondii* according to the method of Choi et al [13]. The optical density (OD) of duplicated samples were measured at 490 nm with a spectrophotometer (PerkinElmer Victor3, Turku, Finland) and compensated by comparing the OD of a standard positive serum in each plate. Comparison between groups was analyzed by the

Student's t-test.

Rapid diagnostic test (RDT)

IgG/IgM RDT mounted with recombinant fragment of major surface antigen (SAG1), GST-linker-SAG1A [14,19], 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 after 15-20 min and its density was determined arbitrarily as 0, +, ++ and +++. A 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

Seroprevalence

The positive seroprevalence of the residents in Gyodong-do surged from 14.7% (135/921) in 2010 to 23.1% (231/993) in 2011 by ELISA. Subsequent years showed an increasing trend in the positive seroprevalence, to 23.6% (222/940) and 32.1% (269/838) in 2012 and 2013, respectively (Table 1; Fig. 2).

Table 1. Seroprevalence of *T. gondii* by ELISA and RDT among residents in Gyodong-do (Island), Gangwha-gun, Incheon, Korea, 2010-2013

Year	No. of sera	ELISA (%)	RDT (%)	ELISA only	ELISA/RDT	RDT only
2010	921	135 (14.7)	200 (21.7)	19	116 (85.9)*	84
2011	993	231 (23.1)	253 (25.5)	28	203 (87.9)	50
2012	940	222 (23.6)	272 (28.9)	24	198 (89.2)	74
2013	838	269 (32.1)	277 (33.1)	39	230 (85.5)	47

*% of ELISA positive which are detected also by RDT.

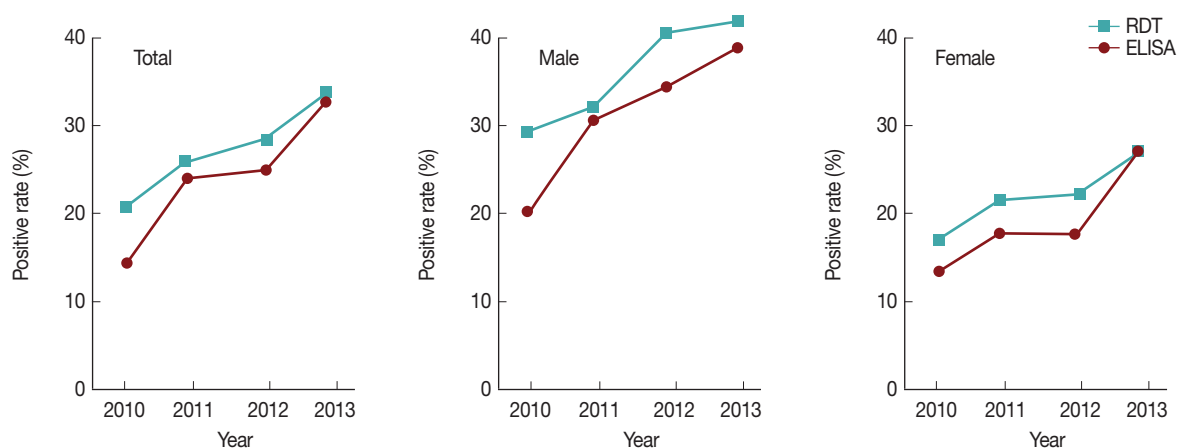


Fig. 2. Trends in positive seroprevalence rate according to gender in the surveyed area.

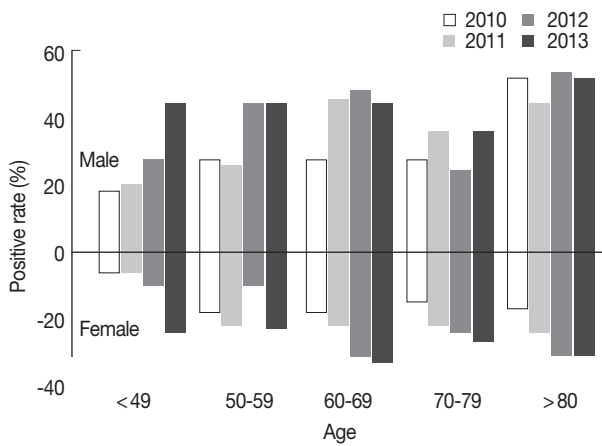


Fig. 3. Comparison of the positive rates of anti-Toxoplasma antibody by gender and age. The positive rates at all age groups in women showed a gradual increase from 2010 to 2013. The same overall increase was observed in men, but surges were observed during 2011-2012 in the age 50's group, and during 2010-2011 in the age 60's group. The seroprevalence at the 70's group was lower than that of the other age groups regardless of gender.

In contrast, it only increased from 21.7% (200/921) in to 25.5% (253/993) in 2011 by RDT. Although a surge was not observed as in the ELISA test, the RDT positive seroprevalence also showed an increasing trend in subsequent years, to 28.9% (272/940) and 33.1% (277/838) in 2012 and 2013, respectively (Table 1; Fig. 2).

The positive rate of men was always higher than that of women, and men showed an explosive surge, whereas it was more gradual in women. Nevertheless, the positive seroprevalence showed a steady increasing trend in both genders (Fig. 2).

As expected, both genders showed lower seroprevalence in the below 50 years old age group. The positive rates at all age groups in women showed a gradual increase from 2010 to 2013. The same overall increase was observed in men, but surges were observed during 2011-2012 in the age 50's group, and during 2010-2011 in the age 60's group. The seroprevalence at the 70's group was lower than that of other age groups regardless of gender (Fig. 3).

Table 2. Discrepancy between ELISA and RDT seroprevalence of toxoplasmosis in Gyodong-do (Island), 2010-2013

Year	OD (%)				
	0.0-0.14 ^a	0.15-0.24 ^a	0.25-0.34 ^b	0.35-0.44 ^b	Over 0.45 ^b
2010	36/685 (5.3%)	48/101 (47.5%)	31/44 (70.5%)	24/29 (82.8%)	61/62 (98.4%)
2011	16/471 (3.4%)	34/283 (12.0%)	31/55 (56.4%)	40/44 (90.0%)	132/132 (100%)
2012	25/414 (6.0%)	49/304 (16.1%)	42/60 (70.0%)	32/38 (84.2%)	124/124 (100%)
2013	9/206 (4.4%)	38/363 (10.5%)	60/97 (61.9%)	44/46 (95.7%)	126/126 (100%)

^aELISA negative, but RDT positive.

^bRDT positive rate in ELISA positive.

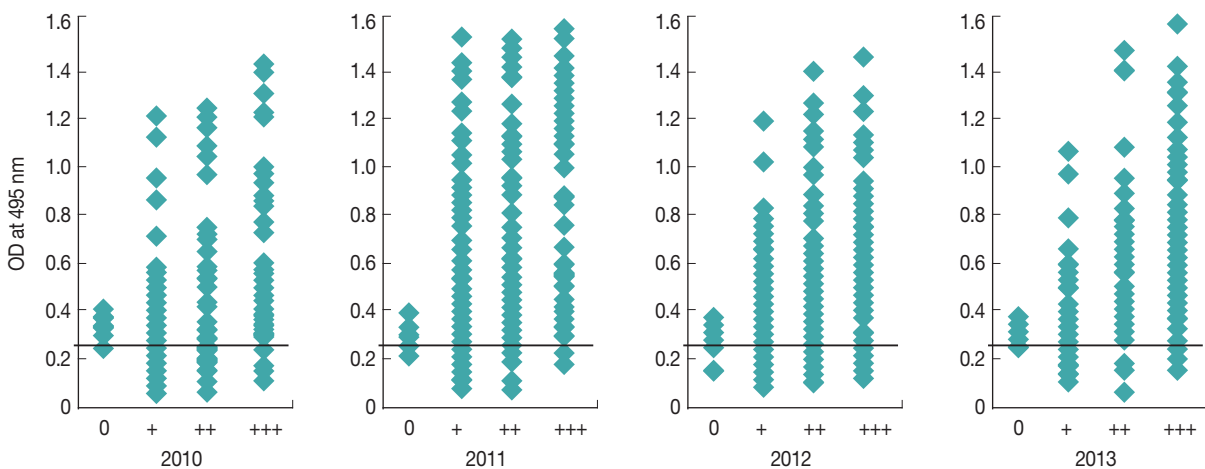


Fig. 4. Comparison of the distribution of positive anti-Toxoplasma antibody titers of the residents in the area. OD line of 0.25 means the positive cut-off of this survey.

Recent sudden surge of toxoplasmosis

In 2010, ELISA detected 135 positives among 921 (14.7%) and RDT detected more sensitively 202 positives (21.9%). However, RDT detected 87.4% (118 sera) of ELISA positives except for those sera of controversial OD just above the cut-off value; instead, it detected more positives in ELISA-negatives of 10.7% (84 sera). This pattern was maintained in 2011, 2012, and 2013 with the increasing seroprevalence of 23.1%, 23.6%, and 32.1% by ELISA and 25.5%, 28.9%, and 33.1% by RDT, respectively (Table 1).

Discrepancy between ELISA and RDT

A cut-off value of 0.25 optical density (OD) at 495 nm was considered positive with the laboratory ELISA kit we used [14]. ELISA values agreed fairly well with RDT results under 0.15 OD and above 0.35 OD, but discrepancies appeared around the 0.15-0.35 range. At the OD 0.15-0.24 range, RDT could detect 16.1% (169/1,051) more positive cases. At the OD 0.25-0.34 range, RDT agreed with ELISA in 64.1% (164/256) of cases (Table 2). Thus, ELISA at this range showed a false positive rate of 35.9% (92/256), which is a rather high value to be comfortable to accept.

Comparison of the distribution of positive anti-*Toxoplasma* antibody titers of the residents in the area showed that a fair number of ELISA negative cases below the OD 0.25 cut-off value were positive by RDT (Fig. 4).

DISCUSSION

Since Soh et al. [20] first reported the seropositive rate of 5.6% in 373 Koreans by skin test using toxoplasmin in 1960, several serological methods, such as ELISA, ILA, and indirect fluorescent antibody test (IFAT) have been developed for detection of *T. gondii* infection (Fig. 5). However, most of these methods need whole cell lysates of *T. gondii* as an antigen which is expensive and time-consuming to prepare. To overcome these disadvantages, we have developed a detection method in the form of rapid diagnostic test (RDT) using recombinant proteins as antigens [19].

The seroprevalence of *T. gondii* has remained fairly stable since 1960 until recently. A major surging increase was observed from 2010 among residents living in Gyodong-do, Incheon, Korea. The RDT results confirmed this trend. The surging increase in the seroprevalence may have been caused by several factors that can reflect environmental and socioeco-

nomical changes in Korea. As Korea is becoming economically ever more affluent, health consciousness is increasing, along with environmental awareness, resulting in the increase in organic agriculture with far less use of pesticides throughout the country [21,22].

The improvement in the environment has caused an increase in the local fauna, thus the potential for *Toxoplasma* hosts has also increased. Our previous studies have been performed around the border of Korea, one of the most naturally preserved areas in the world [23]. Not surprisingly, a relatively high population of local fauna would result in a high seroprevalence of intermediate hosts for *Toxoplasma* [24]. Sometimes they are captured by a snare to be consumed among families and relatives. Furthermore, 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.

In recent years, Korea has seen an alarming increase in the elderly population, and also single-households. This again has caused an explosive increase in pets, and besides the common ones like dogs and cats, exotic animals are also being adopted. These exotic animals are not always obtained legally, and some may harbor diseases [25,26]. Interaction among pets and these feral counterparts are also common in the countryside. Especially troublesome is the increase in feral cats. These cats are

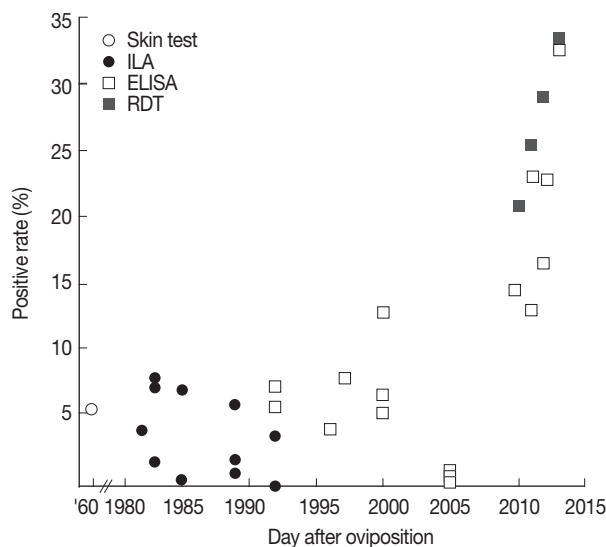


Fig. 5. Trends of positive rates of the surveys of *T. gondii* antibodies in Korea.

ubiquitous in Korea, and they have occupied the top of the food chain in wildlife of Korea with increasing oocyst-shedding rate.

Meat consumption in Korea has increased dramatically recently. Both domestic and imported meat consumption has increased. Combined with the organic fervor, domestic free-range animals are in high demand, which could be another potential route for *Toxoplasma* infection [21,27]. Domestic meat supply is insufficient, so a large amount must be imported. Some of them may have been imported from high endemic nations. Indeed, pork has been imported without any inspection of *T. gondii* [28].

Gangwha-gun is known as the outbreak site of toxoplasmic lymphadenitis by the ingestion of tissue-cysts 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 surging of positive rates on the area, if pork had been served undercooked. These factors, combined with its isolated location may have provided a high worm burden to the inhabitants of the island.

Korea is on the verge of becoming an advanced country with an almost 100% full health-coverage; however, the seroprevalence of toxoplasmosis in certain areas is surging recently. Another factor contributing to the surge may be climatic changes, as the Korean peninsula is experiencing sub-tropical weathers during the warm periods of the year. As a result, communicable disease patterns are also changing, with tropical diseases becoming more common [29,30].

A peculiarity of the RDT needs to be addressed in regard to our study. A cut-off value of 0.25 optical density (OD) at 495 nm was considered positive with the laboratory ELISA kit we used. Our previous study has determined the overall specificity and sensitivity of the RDT to be 100% and 97.1%, respectively [19]. ELISA values agreed fairly well with RDT results under 0.15 OD and above 0.35 OD, but discrepancies appeared around the 0.15-0.35 range (Table 2).

Comparison of the distribution of positive anti-*Toxoplasma* antibody titers of the residents in the area showed that a fair number of ELISA negative cases below the OD 0.25 cut-off value are positive by RDT (Fig. 4). At the OD 0.15-0.24 range, RDT could detect 16.1% (169/1,051) more positive cases. These cases may represent an early or acute toxoplasmosis.

On the other hand, RDT agreed with ELISA in 64.1%

(164/256) of cases at the OD 0.25-0.34 range. This could be interpreted as the ELISA at this range showing a false positive rate of 35.9% (92/256), or RDT having a low detection rate. Both explanations are problematic. ELISA uses *T. gondii* whole cell lysate as the antigen, while RDT uses only rSAG1A-GRA2 antigen. Therefore, the discrepancy may be explained in view of the patient's history. A chronic infection history implies abundant antibodies against various antigens, such as SAGs, GRAs, and MICs, which could be detected by ELISA. RDT would be limited in this respect, as only 2 antigens are detected. As previous studies have suggested, the seroprevalence of this area has been high, so chronic cases would not be rare, which would be detected by ELISA alone, and the recent surge in seroprevalence indicates an increasing incidence of new cases, which would be better detected by RDT. Of course, these discrepancies only appear at the 0.25 cut-off value, and in the majority of the cases, ELISA and RDT agreed on the results. A combined method of using both ELISA and RDT at these levels would be appropriate.

Toxoplasmosis is prevalent throughout the world, in both humans and 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 the seroprevalence, coupled with a change in disease characteristics at any point, may cause a major health threat. As history has revealed, an insignificant retrovirus in the past has caused a near chaotic crisis called AIDS.

It is necessary to find both symptomatic and asymptomatic toxoplasmic patients and confirm the risk factors for further infection in the area. In addition, the problems may not be limited to the island. Indeed, the proposed causes in the study may be applied to a full-scale national survey to screen the national toxoplasmic seroprevalence. In this respect, a national health policy would be required, and with a population of over 50 million in South Korea alone, a rapid and effective screening method will be helpful.

In conclusion, it is necessary to find both symptomatic and asymptomatic toxoplasmic patients and confirm the risk factors for further infection in the area. In addition, 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. Repeated surveys performed on a fixed time interval with more simple method such as RDT to supplement the ELISA is recommend-

ed to define the characteristics of the infection as well as to find new infections.

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CONFLICT OF INTEREST

We have no conflict of interest related to this study.

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