

# A Surge in the Seroprevalence of Toxoplasmosis among the Residents of Islands in Gangwha-gun, Incheon, Korea

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**Abstract:** Seroepidemiological changes of *Toxoplasma gondii* infection among the residents of the islands of Gangwha-gun, Incheon for 2 years were surveyed and evaluated by ELISA using a crude extract antigen. In 2010, sera of 919 adult residents in Gyodong-myeon and 313 adults in Samsan-myeon were collected and checked for IgG antibody titers, which showed 14.5% (133 sera) and 19.8% (62 sera) positive rates, respectively. In 2011, sera of 955 adults in Gyodong-myeon and 341 adults in Samsan-myeon were examined, which showed an increase of positive rates to 23.8% (227 sera) and 31.7% (108 sera), respectively. Totally, the seroprevalence of the first year was 15.8% and it increased rapidly to 25.8% in the second year. The positive rates of both sexes increased simultaneously with the significant ratio of males to females by 1.7-2.2 fold ( $P < 0.05$ ). In both myeons, 661 sera were collected every year and showed changes in optical density (OD) in 177 sera; newly found as positives in 73 persons (11.0%), negative conversion in 10 persons (1.5%), and maintained or increased in 94 persons (14.2%). This rapid increase in the prevalence of toxoplasmosis in Gangwha islands may be due to in part peculiar changes in the toxoplasmic environment of the islands and presumably the consumption of the pork bred domestically within the islands or imported from high endemic nations. It is necessary to find out symptomatic toxoplasmic patients and confirm the risk factors for further infection in the islands of Gangwha-gun.

**Key words:** *Toxoplasma gondii*, seroprevalence, Gangwha-gun, survey, ELISA

## INTRODUCTION

Toxoplasmosis is acquired by the infection with *Toxoplasma gondii*, an apicomplexan zoonotic protozoa, through ingestion of the oocyst form while gardening or consuming fresh products or drinking water contaminated with the oocysts shed in the feces of cats, and more frequently through ingestion of the tissue-cyst form after consuming insufficiently cooked meats carrying the tissue cysts. Another route of infection is from an infected mother to her fetus via placental transmission of *T. gondii*, a process known as congenital toxoplasmosis [1-3]. The congenital toxoplasmosis may cause stillbirth or abortion in addition to serious damages to the fetus, such as severe neurological disorders after delivery [4,5]. Almost all acquired infec-

tions pass by as benign or asymptomatic infections but the infections transform into a chronic status especially in the central nervous system, which sometimes reactivates in immune compromised patients [6] to cause lymphadenitis or ocular toxoplasmosis [7-9].

In the Republic of Korea, there have been many surveys on the seroprevalence of toxoplasmosis among various groups of out-patients to hospitals, which resulted in the range from 1.9 to 5.8% with indirect latex agglutination (ILA) test [10] and up to 7.7% with more sensitive ELISA [11], all of which are still significantly lower by approximately one tenth than those of the other countries of 30-70% [1]. Recently, we have proved the maintained seroprevalence of a patient group and residents in Jeju island over 30 years as 12.9-13.2% [10,12,13], which was higher than the other regions of Korea. In addition, we recently observed a more higher seroprevalence of 17.0% in a survey of residents in Cheorwon-gun located in the northern part of South Korea, which suggests some changes in the toxoplasmic environment [14].

Here, we present results of a survey on the seroprevalence of

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toxoplasmosis among the residents in several villages of islands in Gangwha-gun, Incheon Metropolitan City, localized in the Yellow Sea for 2 years. This is the first report on the seroprevalence of toxoplasmosis in the residents of islands near the mainland Korea and the first survey done 2 times in the same regions at 1 year interval to trace the changes in the prevalence. The seroprevalence of the first year was as high as 15.8% and increased rapidly to 25.8% at the second year, which suggested that the residents of the geographically isolated islands are vulnerable to the infection by risk factors of the toxoplasmic environment.

## MATERIALS AND METHODS

### Parasite and preparation of antigen

The RH strain of *T. gondii* was maintained by peritoneal passages in BALB/c mice. The tachyzoites were purified by centrifugation over 40% Percoll (Sigma-Aldrich Co., St. Louis, Missouri, USA) in PBS [15] 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

In November and December 2010, sera of 919 adult residents (368 males and 551 females) in Gyodong-myeon (Gyodongdo island) and 313 adults (111 males and 202 females) in Samsan-myeon (Seokmodo and Seogeomdo islands) (Fig. 1) were collected. In the same 2 months of 2011, sera of 955 adults (398 males and 567 females) in Gyodong-myeon and 341 adults (male 108 and female 233) in Samsan-myeon were collected. This study was performed under the regulation of the IRB Committee of Chung-Ang University (No. 2010-06-03) held on June 18, 2010.

### ELISA

The sera were checked for IgG antibody titers by ELISA using a crude extract antigen of *T. gondii* (RH) according to the method of Choi et al. [11]. 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.



**Fig. 1.** The surveyed areas. Gyodongdo Island belongs to Gyodong-myeon, and Seogeomdo and Seokmodo Islands belong to Samsan-myeon, both of which belong to Gangwha-gun, Incheon Metropolitan City, Korea.

## RESULTS

The seroprevalence of the residents in 3 islands of Gangwha-gun in 2010 was as high as 15.8% (195 positives among 1,232 sera) and increased rapidly to 25.8% (335 positives among 1,296 sera) after 1 year. The positive rate of the residents in Gyodong-myeon (Gyodongdo island) increased from 14.5% (133 positives among 919 sera) to 23.8% (227 positives among 955 sera) and those in Samsan-myeon (Seokmodo and Seogeomdo islands) from 19.8% (62 positives among 313 sera) to 31.7% (108 positives among 341 sera), respectively (Table 1). The positive rate of males was always higher than that of females in both myeons; however, the ratio of positive rates for males to females was lowered such that the ratio in Gyodong-myeon changed from 1.94 to 1.78 and from 2.20 to 1.66 in Samsan-myeon during the 2 years. There was no regional tropism of the increase in the positive rates within the islands. Regionally, the positive rates at Gogu-ri, Nanjung-ri, Dongsan-ri, and Jiseok-ri of Gyodong-myeon were maintained for 2 years regardless of the positive rates in 2010, while those of the other villages showed more rigorous increases in the positive rates. This was also observed in the villages of Samsan-myeon except for Seokpo-ri and Seogeom-ri.

**Table 1.** Seropositive rates of toxoplasmosis among the residents of Gangwha-gun, Incheon, according to region and sex

Region	2010			2011		
	Male	Female	Total	Male	Female	Total
Gyodong-myeon						
Gogu-ri	10/41 (24.4) <sup>a</sup>	9/64 (14.1)	19/105 (18.1)	5/36 (13.9)	8/64 (12.5)	13/100 (13.0)
Nanjung-ri	4/38 (10.0)	7/63 (11.1)	11/101 (10.9)	4/41 (9.8)	6/69 (8.7)	10/110 (9.1)
Daeryong-ri	3/32 (9.4)	2/52 (3.8)	5/84 (6.0)	7/34 (20.6)	10/70 (14.3)	17/104 (16.3)
Dongsan-ri	4/22 (18.2)	1/30 (3.3)	5/52 (9.6)	4/22 (18.2)	1/30 (3.3)	5/52 (9.6)
Muhak-ri	5/16 (31.3)	6/44 (13.6)	11/60 (18.3)	18/23 (78.8)	11/38 (28.9)	29/51 (56.9)
Bongso-ri	6/33 (18.2)	7/46 (15.2)	13/79 (16.5)	10/27 (37.0)	10/35 (28.6)	20/62 (32.3)
Samsun-ri	9/66 (13.6)	5/73 (6.8)	14/139 (10.1)	19/73 (26.0)	11/68 (16.2)	30/141 (21.3)
Seohan-ri	6/31 (19.4)	5/44 (11.4)	11/75 (14.7)	6/42 (14.3)	16/55 (29.1)	22/97 (22.7)
Yanggab-ri	8/29 (27.6)	3/40 (7.5)	11/69 (15.9)	15/31 (48.4)	11/44 (25.0)	26/75 (34.7)
Eupnae-ri	1/10 (10.0)	1/22 (4.5)	2/32 (6.3)	8/15 (53.3)	4/30 (13.3)	12/45 (26.7)
Insa-ri	1/20 (5.0)	4/37 (10.8)	5/57 (8.8)	12/24 (50.0)	7/34 (20.6)	19/58 (32.8)
Jiseok-ri	18/30 (60.0)	8/36 (22.2)	26/66 (39.4)	18/30 (60.0)	6/30 (20.0)	24/60 (40.0)
Sub-total	75/368 (20.4)	58/551 (10.5)	133/919 (14.5)	126/398 (31.7)	101/567 (17.8)	227/955 (23.8)
Samsan-myeon						
Maeum-ri	3/28 (10.7)	3/54 (5.6)	6/82 (7.3)	9/31 (29.0)	8/67 (11.9)	17/98 (17.3)
Sang-ri	1/5 (20.0)	3/16 (18.8)	4/21 (19.0)	5/6 (83.3)	7/18 (38.9)	12/24 (50.0)
Seogeom-ri	6/16 (37.5)	3/17 (17.6)	9/33 (27.3)	6/18 (33.3)	5/24 (20.8)	11/42 (26.2)
Seokmo-ri	10/28 (35.7)	7/45 (15.6)	17/73 (23.3)	11/23 (47.8)	14/52 (26.9)	25/75 (33.3)
Seokpo-ri	10/22 (45.5)	3/40 (7.5)	13/62 (21.0)	5/11 (45.5)	6/35 (17.1)	11/46 (23.9)
Ha-ri	4/12 (33.3)	9/30 (30.0)	13/42 (31.0)	11/19 (57.9)	21/37 (56.8)	32/56 (57.1)
Sub-total	34/111 (30.6)	28/202 (13.9)	62/313 (19.8)	47/108 (43.5)	61/233 (26.2)	108/341 (31.7)
Total	109/479 (22.8)	86/753 (11.4)	195/1,232 (15.8)	173/506 (34.2)	162/800 (20.3)	335/1,296 (25.8)

<sup>a</sup>percentage of positives.

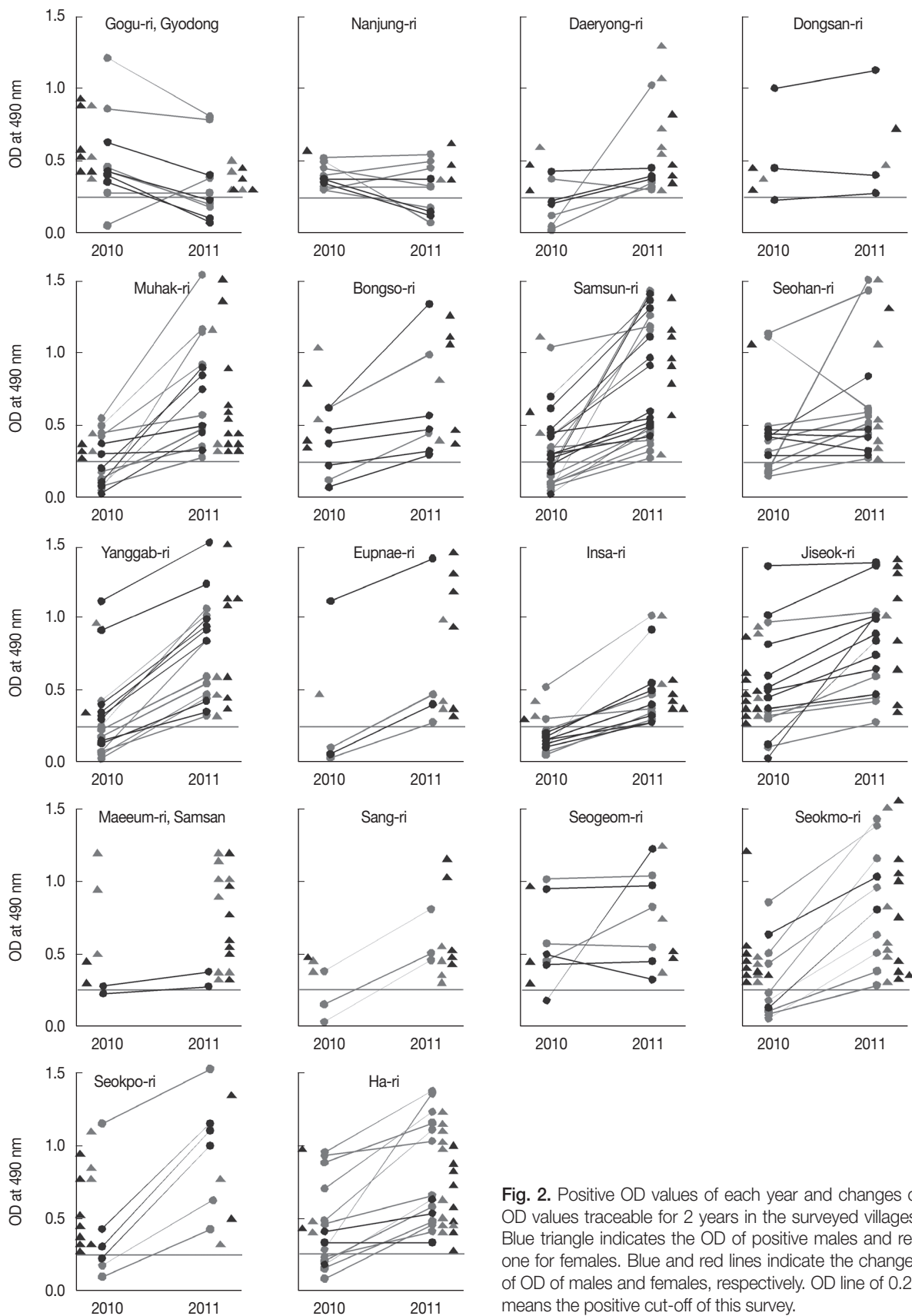
The OD values in the positive criteria of 195 cases in 2010 and 335 cases in 2011 in both myeons were spotted as OD values by year in addition to 73 negative spots in 2010 which were converted to positive in 2011 and 10 positives in 2010 which were converted negative in 2011 according to the regions (Fig. 2). Among the 661 sera collected twice in each year in both myeons, 177 sera were traced for the changes in OD values over the cut-off value, which showed newly found positives in 73 sera (11.0%), negative conversion in 10 sera (1.5%), and maintained or increased in 94 sera (14.2%). Most negative conversions occurred at Gogu-ri and Nanjung-ri residents, sustained positive OD's for 2 years were maintained at Gogu-ri, Nanjung-ri, Dongsan-ri, Maeup-ri, and Seogeom-ri residents, while in the other villiages almost all positive OD values increased after 1 year in addition to seroconversion to positives especially in Daerong-ri, Muhak-ri, Samsun-ri, Yanggab-ri, Insa-ri, Seokmo-ri, Seokpo-ri, and Ha-ri residents.

The positive rates by age of male residents in Gyodong-myeon in 2010 were approximately equivalent to the mean value over all ages from thirties but increased in over sixties in 2011, while those of females were high only in fifties and sixties in

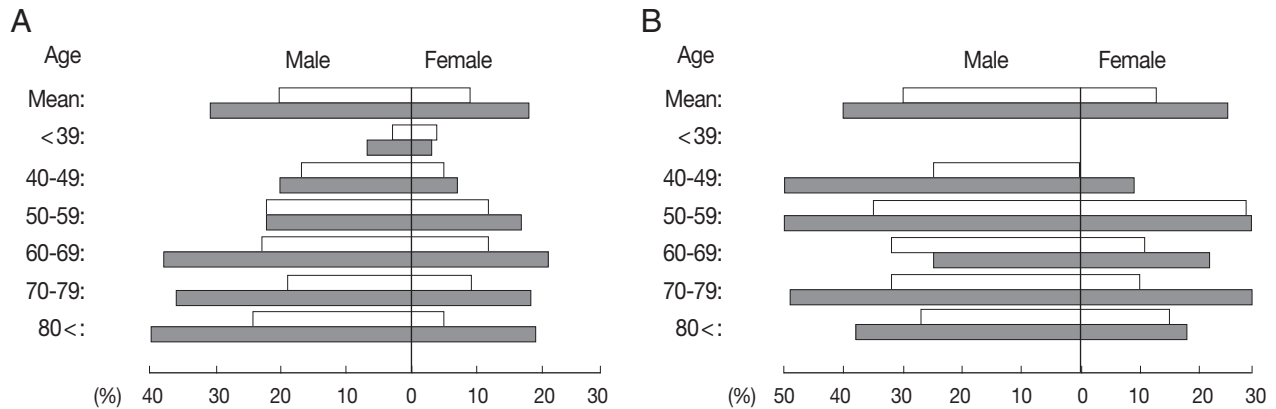
2010 but increased over fifties to eighties (Fig. 3A). In Samsan-myeon, the positive rates over forties were approximately similar to the mean value in 2010 but rapidly increased in all ages except sixties, while the positive rate was high only in fifties in 2010 but increased over all ages (Fig. 3B). Furthermore, the positive OD values were distributed more widely to high OD in females than in males in 2010, while those were distributed more widely to high OD in males than in females reversely in 2011, being still more widely in both sexes than in 2010 (Fig. 4). The median OD values were 0.42 in males and 0.48 in females and the mean OD was 0.51 and 0.58 in 2010, respectively, which increased to median OD values of 0.58 in males and 0.53 in females with the mean OD of 0.72 in males and 0.67 in females.

## DISCUSSION

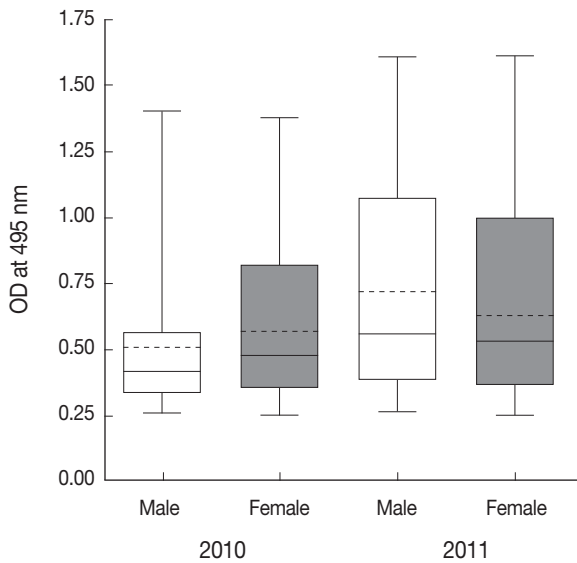
Through the 2 times surveys over 1 year interval on the prevalence of toxoplasmosis among the residents at the same 3 islands of Gangwha-gun, we observed relatively high positive rates across the villages at the first year compared to the previ-



**Fig. 2.** Positive OD values of each year and changes of OD values traceable for 2 years in the surveyed villages. Blue triangle indicates the OD of positive males and red one for females. Blue and red lines indicate the changes of OD of males and females, respectively. OD line of 0.25 means the positive cut-off of this survey.



**Fig. 3.** Comparison of the positive rates of anti-*Toxoplasma* antibody by sex and age. A, Gyodong-myeon; B, Samsan-myeon. Blank bars indicate the positive rates in 2010 and solid bars those in 2011.



**Fig. 4.** Comparison of the distribution of positive anti-*Toxoplasma* antibody titers of the residents in the islands of Gangwha-gun. Solid line indicates the median value and dotted line shows the mean value.

ous screenings in Korea and more importantly a surgical increase in the positive rates for a short time of 1 year. Those newly infected and newly found positives were scattered all over the islands and were more frequent among the families and relatives socially, which strongly suggests that the cause of infection may be food-borne.

The sera of 519 of 919 residents in Gyodong-myeon in 2010 (519 of 955 residents in 2011) and the sera of 142 of 313 residents of Samsan-myeon in 2010 (142 of 341 residents in 2011), 661 sera in total, were collected from the same persons to trace the changes in OD's. In 177 of 661 sera, there were changes in

OD's, which included 73 (11.0%) new infections, 10 (1.5%) negative conversions, and 94 (14.2%) increases in OD. These percentages could also be applied to 158 positive OD's which were collected only once in 2011 (Fig. 2) with the similar partitions. Presuming with the addresses and the first names of the positive cases, almost all women infections were coupled with their husbands simultaneously (i.e., familial infection). Also, the infections were common among the relatives, which suggests the share of live or undercooked foods of infected materials.

The positive rates by age did not follow the general tendency of gradual increase of prevalence according to the ages (Fig. 3), which suggests a rapid change in the toxoplasmic environment in these islands. Fortunately, because the ages of almost all residents were over forties, similar to the other rural regions of Korea, there seems to be no risk factors for congenital infections at this time. In addition, the positive OD values were distributed more widely in 2011 than in 2010 such that the median OD values were 0.42 in males and 0.48 in females and the mean ODs were 0.51 and 0.58, respectively, in 2010. The median OD values increased to 0.58 in males and 0.53 in females and the mean ODs to 0.72 in males and 0.67 in females. This suggests that some infections are at early stages of infection and the other infections are chronic enough to make the specific antibody fluently.

The increase in the positive rates from 15.8% to 25.8% within a year, both rates of which are regarded as the highest ones among the Korean groups tested, seems to be related to changes in the toxoplasmic environment, especially the eating behavior among the 3 generally recognized causes [16], which include the pet-loving preference and residential mode. Gang-

wha-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 [7]. Stray and wild cats are ubiquitous in Korea, and they have occupied the top of the food chain in the wildlife of Korea with an increasing oocyst-shedding rate [17-19]. Regardless of the pig farm or house pigsty, pigs have been bred under the condition where the cats can approach easily. They are served as popular foods in domestic festivals, wedding receptions, and funeral receptions, which presumably explain the surging increase of the positive rates over so many villages of the islands for a year, if served as undercooked.

Another source of *T. gondii* infection not restricted in such isolated islands but all over the rural and suburban regions in Korea is the wild animals, such as the wild boar, deer, or badger that are prolific enough to be harmful to agriculture and socioeconomics. Sometimes they are captured by a snare to be consumed among families and relatives. Moreover, some Koreans have the misbeliefs that raw viscera of wild or domestic animals, such as the liver, kidney, or gall (bile) has special nutrients for stamina. This may explain the differential sex ratios in the prevalence of *T. gondii* infection in addition to more socioeconomic activities of males than females. However, the ratio of the positive rates for males to females was decreased and the ratio of Gyodong-myeon changed from 1.94 to 1.78 and from 2.20 to 1.66 in Samsan-myeon for 2 years (Table 1), which suggests that this risk factor may not contribute frequently as described above. One more source of infection may include the nationwide available imported pork from countries with high toxoplasmic endemicity from European and Australasian countries. The problem is that the pork is imported without inspection of *T. gondii*. Nevertheless, infection by ingesting oocysts cannot be ruled out because of possible poorer surroundings and relatively indifferent supervision of local water supply facilities in those regions.

Altogether, about one-fourth of the residents in 3 islands of Gangwha-gun were found to be infected with *T. gondii*, and further studies are needed on the epidemiology of *T. gondii* infection and other related diseases of the infected individuals. This survey provided basic data which could be helpful for establishing plans to control toxoplasmic lymphadenitis, retinochoroiditis, and encephalitis [20-22]. Repeated surveys at a certain time interval are recommended to monitor the infection status, including new infections. Furthermore, the proposed reasons for the surge in the prevalence may be applied to the other areas of Korea, and it is necessary to screen the na-

tional toxoplasmic seroprevalence periodically.

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